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THE SYNTHESIS AND SECRETION OF MILK FAT

I. The Time of Milk and Fat Secretion[1]

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The formation and secretion of milk has been the source of much speculation for a long time. Numerous and diverse hypotheses have been advanced from time to time to explain this important phenomenon although but very little fundamental work has been done. Milk fat is one of the important ingredients of milk varying, proportionately, more than any other ingredient, both from a standpoint of quantity and chemical composition. Because of such wide variations the authors became interested in finding the mode of its synthesis and secretion.

In order to make a comprehensive study of the physiology of milk fat secretion and synthesis, it is first necessary to ascertain the time when such activities take place. Textbooks and teachers of dairy science state that the udder of a high producing cow is incapable of containing all the milk that is drawn at a milking. Isaachsen (1923) states that the udder of cows producing 5 to 6 kilos of milk is capable of holding only 3½ kilos and that from 2 to 2½ kilos must be secreted during milking. Nuesch (1904) was one of the first to definitely postulate that milk secretion presents two phases; the first a more or less continuous secretion in the interim between milkings and the second a rapid secretion during the milking process. He attributed the secretion during the milking to a nervous stimulation caused by the milking manipulations.

Maxwell and Rothera (1915) working with cats concluded that about forty per cent of the milk yield was secreted at the time of milking due to

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the mechanical stimulus of sucking. They based this statement upon the estimate of lactose in the glands of cats killed at milking time and upon an estimate that cat's milk contains 5.07 per cent lactose. Gaines (1915) measured by displacement the decrease in the volume of goats' udders due to milking. He found that withdrawal of 339 cc. milk decreased the udder volume 323 cc. Although the author does not call attention to this, it is possible that the decrease in pressure due to the removal of the milk might increase the body fluids in the vascular system of the gland sufficiently to account for the difference between the volume decrease of the gland and the amount of milk withdrawn.

Zwart (1916) was the first to show that the udder of the bovine was capable of containing all the milk yielded at a milking. He was able to inject back into the gland more than was withdrawn and by slaughtering cows at milking time and expressing the milk from the cut up gland recovered very nearly the calculated yield. Gaines and Sanmann (1927) and Gowen and Tobey (1928) corroborated this by using a different technique. They slaughtered cows at the regular milking time and by milking the excised glands and determining the lactose content of the gland were able to account for more milk than was indicated from pre-slaughter records. Use of the lactose content of the gland for estimation of the milk in the gland does not necessarily prove that the milk was present in the natural form as the milk constituents might be present in a concentrated form.

Swett (1927) also found that the excised glands from cows slaughtered at milking time yielded nearly the calculated amount of milk for a normal milking, but noticed that the fat content of the post-mortem milks was lower than normal. Swett accounts for the low fat content of the post-mortem milk by assuming that the fat particles were held back because of the smallness of the milk ductules. Gaines (1915) explained the increase of fat content with the progress of a milking in the same way.

METHOD OF PROCEDURE AND TECHNIQUE. To determine whether or not the mammary gland actually has the capacity for holding all the milk produced at a milking, a number of different methods might have been used. The size of the gland might be determined by displacement before and after milking; the drawn milk might be reinjected; the animal might be slaughtered and the milk content measured after removal from the gland after slaughter, or estimated from the amount of some characteristic and constant milk constituent in the gland, determined by chemical analysis.

The first method mentioned was used by Gaines (1915). It is open to two criticisms. First, because of the anatomical location and shape of the gland it becomes difficult to measure its volume by displacement or any other method. Second, as long as the circulatory fluids pass through the gland the effect upon the capacity of the vascular system of the gland

through the decreasing pressure caused by the removal of the milk is not known, and may be a source of considerable error. The reinjection of the withdrawn milk would determine whether or not the udder has the capacity for holding the total amount produced at a milking but does not necessarily prove that all the milk is present in the udder at the inception of milking.

The method adopted in this study, that of milking the excised gland, is not entirely free from criticism. It is difficult to estimate the amount of milk which an animal should give at any time because of normal fluctuations in the amount produced from time to time.

As it was found necessary to change the method slightly as the work progressed a detailed statement of the method employed is given with the report of each cow. As it was not possible to slaughter animals especially for this experiment, advantage had to be taken of the slaughter of lactating animals for other purposes. For this reason it was not always possible to get the history of individual milkings before slaughter for as long a period as was desired. The first animal was slaughtered at milking time after individual milkings had been studied for five days previous to slaughter, and the post-mortem milkings compared with the previous corresponding milkings. For the other five animals the right gland was milked dry at the time of slaughter and the left gland after slaughter. Study of the previous milkings was carried out wherever possible. In this way a double check was secured on the estimation of the amount that should be produced. The first check is made by comparison with the corresponding preceding milkings and a second check on the probable normalcy of the post-mortem milking is made possible by comparison with the amount produced by the right gland at the time of slaughter.

As soon as the animals were bled the udders were excised, care being taken not to cut into the gland tissue. A slightly larger area of skin than that covering the gland was removed with the udder. Immediately after removal, which never exceeded fifteen minutes after slaughter, the gland was taken to a warm room where it was suspended in as nearly as possible the natural form. Suspension was made possible by means of stout wire hooks through the suspensory ligament and the surrounding skin. The hooks were fastened to a stout net-wire frame which could be tilted so as to give the natural level to the gland.

Immediately after being suspended the drawing of the milk was begun. This was done in the usual manner of milking. As a matter of fact little difference was noted in post-mortem milking as compared to in-vivo milking. The sphincter muscles at the end of the teats apparently retained their tonicity and there was no evidence of congestion due to rigor mortis except that after the milk of the cisterns was removed the milk did not drain as rapidly as in-vivo. For this reason it became necessary to draw

the milk at intervals up to twenty hours after slaughter before the gland was dry.

The post-mortem milkings as well as the in-vivo milkings were drawn in successive portions for sampling for chemical analysis. In all cases butterfat was determined by the Babcock test. In some cases composite samples of the successive portions were taken for the following analyses: total solids, solids-not-fat, sugar, and ash. Total solids and fat were determined by the Mojonnier method in composite samples. Solids-not-fat percentage was calculated by the difference between total solids and the fat. Milk sugar was determined using Fehling's solution according to Richmond (1914).

TABLE 1

Comparison of post-mortem and corresponding pre-slaughter milkings

Mature Ayrshire cow. Slaughtered six weeks after freshening. Slaughtered at milking time 8 hours after preceding milking. Neither side of udder milked before slaughter. Only two fore quarters were milked after slaughter, the rear two being left for microscopic examination. The left half of the udder always produced less than the right half.

	RIGHT FRONT QUARTER		LEFT FRONT QUARTER	
	Amount	Fat	Amount	Fat
	grams	*per cent*	*grams*	*per cent*
March 2, 1926	1,773	5.8	854	4.9
March 3, 1926	1,863	4.9	909	4.9
March 4, 1926	1,683	4.8	899	5.1
Average	1,773	5.17	887	4.95
After slaughter, March 5	1,761	3.7	981	3.9

In all cases the sugar was determined within twenty-four hours after the milk was drawn. The milk was kept frozen to prevent any decomposition of the sugar.

RESULTS. The results of the work on three cows are presented in detail in tables 1, 2, and 3 and the summary of the work for all six cows is presented in table 4. Details are presented for the Ayrshire cow in table 1 because this was the only case in which the post-mortem milking was based only on the records of the corresponding previous milkings. In all other cases the right gland was milked at the time of slaughter and the post-mortem milking of the left gland compared with both the preceding corresponding milkings and the milking of the right gland immediately before slaughter.

The details presented in tables 2 and 3 are typical of the other three experiments, so a summary of the latter will suffice.

As the results of the work on the remaining three cows are typified by

those given somewhat in detail in tables 2 and 3, a general statement about each animal together with the summary given in table 3 will suffice.

Cow E 90 was a mature cow of mixed breeding in an advanced stage of lactation. She was slaughtered June 10, 1927. As with the preceding

TABLE 2

Comparison of post-mortem and in-vivo milkings

Record of common cow E 25. Slaughtered 13 hours after preceding milking. Right gland milked dry at the time of slaughter, left gland after slaughter. October 14, 1926.

Average of two corresponding pre-slaughter milkings

	RIGHT GLAND	LEFT GLAND
Amount milk (grams)	1,901	1,897
Fat (per cent)	3.5	3.6
Total solids (per cent)	11.378	11.560
Solids-not-fat (per cent)	7.878	7.960
Sugar (per cent)	4.32	4.33
Ash (per cent)	0.7420	0.7391

Record of slaughter and post-mortem milkings

PORTION	RIGHT GLAND (MILKED AT SLAUGHTER)		LEFT GLAND (POST-MORTEM MILKING)	
	Amount	Fat	Amount	Fat
	grams	*per cent*	*grams*	*per cent*
1	1,240	2.1	415	2.0
2	539	6.1	393	2.0
3	67	7.2	377	2.2
4			394	2.3
5			285	4.2
6			311	2.2
Total milk (grams)	1,846		2,175	
Average fat (per cent)	3.778		2.405	
Total fat (grams)	63.74		52.32	
Total solids (per cent)	11.279		10.005	
Solids-not-fat (per cent)	7.501		7.60	
Sugar (per cent)	4.431		4.200	
Ash (per cent)	0.7327		0.6845	

cows, each gland was milked separately for three days prior to slaughter and the right gland milked at the time of slaughter and the left gland after slaughter.

Cows designated as X and Y were also of mixed breeding and mature. They were secured from a local slaughtering plant where no history of lactation was available. They arrived at the slaughtering plant on the

afternoon of July 14th and were slaughtered on the morning of the 15th. Each gland was milked separately on the evening of the 14th. As there were no means of knowing when they had been milked previous to arrival, only amounts of milk from the individual glands and the fat percentages were determined. From the distended appearance of their udders it was

TABLE 3

Comparison of post-mortem and in-vivo milkings of E 23

Slaughtered 13 hours after preceding milking. Right gland milked dry at the time of slaughter; left gland after slaughter. October 14, 1926.

Average of two corresponding pre-slaughter milkings

	RIGHT GLAND	LEFT GLAND
Amount milk (grams)	1,739	1,802
Fat (per cent)	5.2	4.9
Total solids (per cent)	14.000	13.9332
Solids-not-fat (per cent)	8.800	8.8332
Sugar (per cent)	4.408	4.449
Ash (per cent)	0.7140	0.7220

Record of slaughter and post-mortem milkings

PORTION	RIGHT GLAND (MILKED AT SLAUGHTER)		LEFT GLAND (POST-MORTEM MILKING)	
	Amount	Fat	Amount	Fat
	grams	*per cent*	*grams*	*per cent*
1	758	2.9	370	2.0
2	807	8.0	351	2.0
3	81	11.0	358	2.2
4			412	5.5
5			63	9.1
6			265	3.2
Total milk (grams)	1,646		1,819	
Fat (per cent)	5.79		3.25	
Total fat (grams)	95.45		59.16	
Total solids (per cent)	14.61		12.34	
Solids-not-fat (per cent)	8.82		9.07	
Sugar (per cent)	4.418		4.125	
Ash (per cent)	0.707		0.691	

apparent that they had not been milked for some time previous to their arrival, and it was thought that further chemical analysis of the milk would be of no value. All four quarters of both udders appeared normal and sound, which is borne out by the fact that milk yields of the right and left glands were nearly identical.

DISCUSSION OF RESULTS. A study of table 4 reveals that four out of the

five cows, where the right half of the udder was milked dry before slaughter, produced more in the post-mortem milking of the left half than in the slaughter milking of the right half. The cow X, whose previous history is unknown, produced slightly less as did number 11 on the right fore quarter, which is compared to the average of the three preceding corresponding milkings. (It has previously been noted that in the case of number 11 neither half of the udder was milked at the time of slaughter.) The post-mortem milking of the left gland of number 11 was considerably larger than the average of the three preceding corresponding milkings.

TABLE 4

Summary of milk and fat yields of the post-mortem milkings and corresponding in-vivo milkings

cow	RIGHT GLAND						LEFT GLAND					
	Average of corresponding milkings before slaughter			Milking at slaughter			Average of corresponding milkings before slaughter			Post-mortem milking		
	Amount milk	Fat	Fat yield	Amount milk	Fat	Fat yield	Amount milk	Fat	Fat yield	Amount milk	Fat	Fat yield
	grams	*per cent*	*grams*	*grams*	*per cent*	*grams*	*grams*	*per cent*	*grams*	*grams*	*per cent*	*grams*
17	773	5.17	82.66	1,761*	3.7	65.16	887	4.95	43.91	981	3.9	38.26
E 25	1,901	3.5	66.57	1,846	3.78	63.74	1,897	3.6	68.29	2,175	2.41	52.32
E 23	1,739	5.2	89.43	1,646	5.79	95.45	1,802	4.9	88.30	1,819	3.25	59.16
E 90	1,407	4.0	56.28	1,519	3.8	57.72	1,362	3.9	53.12	1,455	2.83	41.31
X	3,314	4.3	142.5	1,884	4.1	77.24	3,405	4.8	163.44	1,830	3.54	64.85
Y	2,769	5.8	160.6	1,213	5.4	65.5	2,533	6.2	157.05	1,282	3.25	41.62

* The right gland of 17 was not milked at slaughter but after slaughter.

The milking before slaughter for E 90, X and Y was the evening milking rather than the corresponding milking before slaughter.

One of the interesting facts is that in every case the fat percentage and total yield of fat in the post-mortem milkings are noticeably smaller than in the in-vivo milkings. It is also noteworthy that where the post-mortem milking was sampled as the milking progressed, the fat percentage rises to a maximum and then drops to almost the level of the first portion. This is in contrast to the in-vivo milkings where the last drawn portion has the highest fat content.

Only in two cases were the total solids, sugar and ash content determined. These determinations were made from composite samples consisting of aliquot parts from the different portions of the milkings. In each case, as revealed by tables 2 and 3, these constituents were slightly lower for the post-mortem milking than for the in-vivo milking. As the fat

content of the last drawn portion of the post-mortem milking is very low and the secretion appeared more watery than the earlier drawn portions, it is possible that the last portion is responsible for lowering the solids-not-fat.

The results herein reported differ from those of Swett (1926) in that a greater amount of post-mortem secretion was secured and also that the solids-not-fat content was slightly lower (comparatively). The latter has been partially explained in the last paragraph. The former can be explained by the fact that the author continued the milking over a longer period of time. Undoubtedly rigor mortis begins to set in shortly after death creating a condition which hinders the passage of the milk downward thus requiring a longer time than in life. It was at first thought that this condition was also responsible for the lower fat content of the post-mortem milking. However, the idea was abandoned when microscopic examination showed no more fat particles in the ductules in sections from the left gland than from the right gland. Nor can reasoning permit of the thought that the fat particles are held back in the ducts and the liquid portion permitted to flow by. Normally the fat particles are spherical and the ductules are cylindrical. Should the ducts physically retard the progress of the fat particles it would seem reasonable to believe that these obstructed particles would occlude the flow of the liquid.

SUMMARY AND CONCLUSIONS

A study of the time of milk and fat secretion on six cows is reported. One was slaughtered at milking time, the udder removed and milked after being suspended. With five cows the right gland was milked at the time of slaughter and left gland after removal after slaughter. Comparisons are made of the post-mortem milking with the in-vivo milkings for amounts of milk and fat percentage for all six cows and for sugar, ash, and total solids for two cows.

As either more or nearly as much milk was secured in the post-mortem milkings as in the in-vivo milkings, it is concluded that the cow's udder not only can but does contain practically all the milk produced at a milking. The same fact shows that in all probability the milk is secreted at a more or less constant rate in the interim between milkings. This conclusion is contrary to the general belief that a greater or less portion of the milk drawn at a milking is secreted at milking time.

The much lower fat content of the post-mortem milking lends support to a hypothesis that milk fat is secreted by a separate mechanism from the one responsible for the secretion of the other ingredients of milk.

The slightly lower solids-not-fat content of the post-mortem milkings is not significant. This may be caused by dilution with water produced by dehydration of the tissue cells, which finds support in two facts, 1, the

last post-mortem milkings appeared more watery than the first; 2, there was a decline in the fat percentage.

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THE SYNTHESIS AND SECRETION OF MILK FAT

II. An Analytical Study of the Fat of the Bovine Mammary Gland[1]

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Microscopic studies of histological sections of the lactating bovine mammary gland showed the presence of large amounts of fat. The uniformity with which this fat was dispersed throughout the gland tissue give reason to believe that it was not mere adipose fat for it was seldom visible macroscopically. This suggested the possibility that this fat is concerned in the synthesis and secretion of milk fat. The first problem was to determine the amount of fat in glands at various stages of the lactation period; the next, to determine the character of the fat in the gland tissue as compared with milk fat and body fat.

Literature. At the time this work was started (March 1926) there were no records in the literature on the analysis of cow's mammary glands. Recently two papers concerning this have appeared. Laxa (1927) analyzed the glands of nine cows of unknown origin. The water content varied from 72.7 to 79.9 per cent, and fat from 4.97 to 14.13 per cent. On the dry basis the fat varied from 24.5 to 52.35 per cent. For one gland, of which no description is given, the various fat constants were determined on the ether extract. One portion of the gland was immediately washed in boiling water while the other was not. For both portions he reports a relatively high Reichert-Meissl number, with a lower saponification number for the portion washed in boiling water. Gowan and Tobbey (1928) report Reichert-Meissl numbers for gland fat of two lactating cows to be 11.8 and 14.4 and for non-lactating cows to vary from 0.0 to 0.4.

Material and sources. For this investigation glands were secured from cows in various stages of lactation that were slaughtered at University Farm and a nearby slaughtering plant. Glands were studied from cows in the first ten days of lactation up to one which had been "dry" for over one year.

[1] The data in this paper are taken from a thesis presented to the Graduate Faculty, University of Minnesota by W. E. Petersen in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Published with the approval of the Director as Paper no. 852, Journal Series, Minnesota Experiment Station.

METHOD OF PROCEDURE AND TECHNIQUE. With the exception of the glands used for post-mortem milkings, all glands were removed to a cold temperature room immediately after slaughter, where they were frozen solid at a temperature varying from 0° to −12°F. Glands which were first used for post-mortem milking experiments were frozen immediately after the milkings were completed. All glands were kept in the frozen state until analysis was started. They were then removed to a warm room to permit thawing of the outer layers so that the skin and subcutaneous non-glandular layers could be removed. It was found much easier to dissect off the non-glandular tissue when the glands were in the frozen state.

After the outer layers of non-glandular tissue had been carefully and completely removed, a section of each half of the glands was sawed out for microscopic study. This section was kept below 0°F. until used. The rest of each gland was cut into narrow strips and passed through a food chopper ten times while still in a frozen or semi-frozen state. From the thoroughly mixed ground material samples were taken for the following determinations: dry matter, ash, and ether and alcohol soluble matter. What was left was thoroughly dehydrated by mixing with calcium sulphate and immediately passing through a food chopper several times. The hydrated material was placed in small muslin bags and extracted with ether in a continuous extractor for several days.

The ether extract was filtered after a portion of the ether had been evaporated off, any remaining ether and moisture in the extract were removed under reduced pressure at a temperature of 80°C. The residue constituted the ether extract used for a study of the chemical composition.

The fat immediately surrounding the glandular tissue and just inside the skin was regarded as adipose fat. In some cases it was rendered by heating in a hot water bath and squeezing out the fat through a cheese cloth and then filtering on a hot filter. In other cases the fatty tissue was dehydrated with calcium sulphate and extracted with ether as for the glandular tissue. No difference in the chemical composition was noticed that could be attributed to the method of extraction of the fat.

Moisture determination of organic compounds can only be approximate, for it is well known that organic matter will disintegrate before the last traces of moisture are driven off. It is particularly difficult to determine moisture on material like the ground mammary gland. No matter how many times the material was passed through the food chopper there remained fairly large particles due to the large amount and fine distribution of connective tissue. The method adopted was to plaster as thin a layer of the ground material as possible upon a watch glass and dry in an oven at 100° C. for six hours. About ten grams of material were used for each determination. While the figures used were those secured after the

six hour drying all samples were dried for a longer time and only very slight losses of weight were noted.

For quantitative determination of the ether soluble material, ten grams of the ground material were dehydrated by mixing with calcium sulphate and grinding in a mortar and extracted in a small Soxhlet extraction apparatus for three days. After the experiment had progressed it was found desirable to determine the cholesterol and organic phosphorus content of the gland tissue. For these determinations ten grams of ground tissue were dehydrated with calcium sulphate and extracted alternately with ethyl ether and absolute alcohol. This extraction was done in the Soxhlet extraction apparatus for four days.

CHEMICAL METHODS USED. On all ether extract residues the following determinations were made: iodine number, Reichert-Wollny number and saponification number. These determinations were made according to the directions of Lewkowitch (1909), using Hübl's method for iodine number.

Cholesterol was determined on the ether-alcohol extract by the Meyers and Wardell (1918) method. Organic phosphorus was determined on another fraction of the ether-alcohol extract according to Bauman (1924) except it was found necessary to use more perhydrol than he recommends and to add ferric cyanide as a catalyst. At first considerable difficulty was experienced in getting complete oxidation. This was overcome by heating for one hour at a temperature not high enough to liberate SO_3 fumes and then to heat according to Bauman's directions.

ANIMALS USED IN THIS EXPERIMENT. No. 19, a mature Ayrshire cow, was slaughtered March 5, 1926. At the time of slaughter she had been in milk six weeks. Dissection showed an unusual thick layer of fat surrounding the gland tissue.

E 90, slaughtered June 11, 1926, was a common cow, previous history unknown, except that she was in an advanced stage of lactation and suffered from a phosphorus deficiency. As contrasted with no. 19, there was very little subcutaneous fat surrounding the glandular tissue.

E 25, slaughtered October 14, 1926, was a grade Holstein cow in milk 10 months and in good state of nutrition. Only a moderate amount of subcutaneous fat was found surrounding the glandular tissue.

E 23, slaughtered October 14, 1926, had been in milk 11 months. Gland similar to E 25.

No. 310, slaughtered October, 1926, because of sterility, was a 14 year old Holstein cow. She had been dry six weeks at the time of slaughter. Her udder shortly after freshening was the largest of any in the University herd. It probably would weigh over 80 pounds at that time. At the time of slaughter the gland had shrunk to only a fraction of the size at the time of freshening. She was in an excellent condition of flesh.

No. 101, slaughtered October, 1927, was a 17 year old Jersey cow, dry for one year prior to slaughter and farrow. No. 101 was known for her large udder of good quality when in milk. She was the heaviest producer of the Jerseys in the University herd. At the time of slaughter her gland was only a fraction of the size when in full milk. In the latter condition the gland would weigh over 60 pounds. At the time of slaughter 101 was suffering from arthritis but otherwise was in good health and in fair condition of flesh.

Glands from cows X and Y were secured from a nearby slaughtering plant, and no previous history was available. Apparently, they were in an advanced stage of lactation.

TABLE 1

Dry matter and ether extract in glands

COW	LACTATION PERIOD	DRY MATTER		ETHER EXTRACT			
				Wet basis		Dry basis	
		Right gland	Left gland	Right gland	Left gland	Right gland	Left gland
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
19	In milk 6 weeks	25.8	25.0	13.39	13.39	51.90	53.56
E 90	Advanced	23.4	23.8	7.49	7.73	32.01	32.56
E 23	In milk 11 months	21.64	23.18	9.84	9.76	45.47	42.05
E 25	In milk 10 months	23.00	24.90	8.40	9.42	32.17	33.01
310	Dry	20.44		2.05		10.03	
101	Dry	22.30		6.82		30.58	
E 91	Dry	19.34		4.25		21.98	
X	Unknown	25.40	24.50	10.20	11.00	40.16	44.89
Y	Unknown	24.15	23.6	9.45	9.10	39.13	38.56
Averages:							
For lactating glands		23.90	24.16	9.80	10.07	40.14	40.77
For dry glands		21.03		4.37		20.86	

In case of no. 17, only the front quarters were analyzed, these being milked dry after slaughter. For all other cows in milk, the right gland was milked dry before slaughter and the left gland after slaughter.

RESULTS OF ANALYSIS. *Dry matter and ether extract.* Table 1 gives the percentage ether extract on both wet and dry basis and the percentage dry matter. For the cows in milk separate determinations were made for the right and left halves of the gland while for dry cows only one-half was analyzed. From table 1 it is noted that there is considerable variation between the various glands in their ether extract content as well as the amount of dry matter.

The three glands from dry cows are noticeably lower in fat content than the glands from the cows in milk. The average dry matter is also less for

the dry glands than for those in milk. The high fat content of no. 19 is noteworthy because of the pronounced meaty condition of the gland in life, also that she had been in milk for the shortest period of any of the cows slaughtered.

The high fat content of the mammary gland on the dry basis is especially significant. Only in two cases was fat macroscopically visible when the glands were dissected. These were nos. 17 and 101. In case of 101 the fat formed small pellets on the stirring rod when the ground tissue was mixed.

For the cows, E 90, E 23, and E 25, the milk was also analyzed, and it is of interest to note the close correspondence between the fat content of the dry gland and the proportion of fat in the milk solids as shown by the following data.

Fat percentage of dry matter

	MILK	GLAND TISSUE
	per cent	*per cent*
E 90	30.83	32.28
E 23	39.63	43.76
E 25	32.49	32.59

Table 2 gives the total net weight of each gland with the calculated dry matter and fat. The net weight of gland tissue was determined by weighing the gland after all non-glandular tissue had been dissected away. This table shows the dry glands to contain much less dry matter and fat than those in milk, indicating a marked atrophy of the gland as lactation ceases. The fact that fat decreases more than dry matter is noteworthy as both of the cows from which the dry glands came were gaining in weight.

TABLE 2

Weights of gland tissue with total dry matter and fat

COW	LACTATION PERIOD	TOTAL WEIGHT	TOTAL DRY MATTER	TOTAL FAT
		kgm.	*kgm.*	*kgm.*
19	In milk 6 weeks	18.160	4.612	2.331
E 90	Advanced	5.539	1.308	0.422
E 23	In milk 11 months	5.766	1.294	0.570
E 25	In milk 10 months	6.946	1.663	0.618
310	Dry 6 weeks	3.178	0.650	0.065
101	Dry 1 year	2.724	0.607	0.186
X	Unknown	6.810	1.701	0.710
Y	Unknown	6.901	1.647	0.640

Iodine number. Table 3 gives the iodine number of the ether extract of the glandular tissue, the adipose fat taken from around the glandular tissue, and of the milk fat. The latter was determined in two cases only.

It will be seen that the iodine number varies greatly with the individual. In case of E 90 the ether extract from the right half had an iodine number of 49.8, while from the left half it was 41.4. For the other cows the iodine number for the two halves of the gland checked more closely. The iodine number of the fat from 101 and 310, the two dry glands, is higher than for the glands in milk. In both of these cases the figures are higher for the gland fat than for the adipose fat. In all cases where the cows are in milk the reverse is true.

TABLE 3

Iodine numbers of gland tissue fat, milk fat and adipose fat

COW	LACTATION PERIOD	UDDER FAT		MILK FAT		ADIPOSE FAT
		Right half	Left half	Right half	Left half	
19	In milk 6 weeks	30.4	29.7	—	—	47.0
E 90	Advanced	49.8	41.4	—	—	63.1
E 23	In milk 11 months	36.8	37.8	33.3	36.8	51.4
E 25	In milk 10 months	40.4	40.6	36.8	38.6	45.0
310	Dry 6 weeks	57.5	—	—	—	49.9
101	Dry 1 year	51.0	—	—	—	45.3
X	Unknown	38.9	37.7			44.2
Y	Unknown	38.3	35.5			53.8
Averages:						
For lactating glands		39.1	37.1			50.7
For dry glands		54.3				47.6

Attention is called to the variation in iodine value of the adipose fat; for E 90, E 23, 310, 19 and X it exceeds the normal limits as given by Lewkowitsch (1909). The iodine value of the gland fat from the two cows not in milk also exceeds the normal limits of beef tallow. The iodine value for the fats from the lactating glands is much lower, being on the average between the normal values for butterfat and beef fat.

The Reichert-Wollny number. Table 4 showing the Reichert-Wollny number of the gland fats and the adipose fats brings out the fact that all gland fats have appreciable volatile fatty acid content. It is noted that fats from the lactating glands have a much higher Reichert-Wollny number than those from the non-lactating glands. For all lactating glands, except Y, the left half has a higher Reichert-Wollny value than the right half. This is undoubtedly due to the retention of milk fat in the left half which was milked after slaughter. It has been noted, previously, that in

post-mortem milkings part of the milk fat was retained in the gland. For E 23, however, the difference between the Reichert-Wollny value of the right and left half is too great to be accounted for by the calculated amount of milk fat retained. Some other unexplained factor must have entered in.

TABLE 4

Reichert-Wollny number of gland tissue fat, milk fat and adipose fat

COW	LACTATION PERIOD	UDDER EXTRACT		MILK FAT	ADIPOSE FAT
		Right half	Left half		
19	In milk 6 weeks	5.85	6.50		0.3
E 90	Advanced	7.35	7.85		0.2
E 23	In milk 10 months	5.80	13.00	25.40	0.4
E 25	In milk 11 months	7.25	9.95	23.05	0.5
310	Dry	1.80			0.3
101	Dry	2.95			0.4
X	Advanced	10.20	11.00		0.65
Y	Advanced	9.45	9.10		0.39
Averages:					
For cows in milk		7.65	9.57		0.41
For dry cows		2.38			0.35

TABLE 5

Saponification number of gland tissue fat, milk fat and adipose fat

COW	LACTATION PERIOD	UDDER EXTRACT		MILK FAT		ADIPOSE FAT
		Right half	Left half	Right half	Left half	
19	In milk 6 weeks	207.5	210.9			195.3
E 90	Advanced	209.9	212.7			196.6
E 23	In milk 11 months	214.6	212.2	228.4	228.3	196.2
E 25	In milk 10 months	207.1	211.9	219.4	218.9	196.0
310	Dry	197.9				195.3
101	Dry	193.4				197.2
X	Advanced	209.4	210.6			198.9
Y	Advanced	212.8	213.8			192.7
Averages:						
For cows in milk		210.2	212.0			195.95
For dry cows		195.7				196.25

Saponification number. From table 5 it is seen that the saponification number of the fat from glands in milk is considerably higher than from the dry glands. The saponification number from the dry glands is about normal for body fat while from the glands in milk about intermediate in value between body fat and milk fat. As with the Reichert-Wollny number

the saponification number is usually higher for the left gland, which was milked after slaughter.

Organic phosphorus and cholesterol. The results of the organic phosphorus and cholesterol determinations for two glands are given in table 6. Organic phosphorus, as secured in an absolute alcohol-ether extract, presumably comes from phospholipoids. Table 7 shows the percentage of lecithin in the gland and in the gland fat, assuming that all the organic phosphorus came from this source, and using 777.93 as the molecular weight of lecithin. This is purely hypothetical as it is not known that lecithin, however, serves the point here, namely, that the phospholipoid lecithin is the only phospholipoid present. Conversion of the phosphorus

TABLE 6

Organic phosphorus and cholesterol content of alcohol-ether extract from gland tissue

COW	PHOSPHORUS PER 100 GRAMS TISSUE		CHOLESTEROL PER 100 GRAMS TISSUE	
	Right gland	Left gland	Right gland	Left gland
	mgm.	*mgm.*	*mgm.*	*mgm.*
E 23	6.725	7.264	185.5	167.6
E 25	6.068	7.491	210.0	185.0

TABLE 7

Calculated lecithin percentage on the basis of organic phosphorus content

COW	RIGHT GLAND		LEFT GLAND	
	Of the moist gland	Of the fat	Of the moist gland	Of the fat
	per cent	*per cent*	*per cent*	*per cent*
E 23	0.170	1.73	0.182	1.86
E 25	0.152	1.79	0.187	1.99

into terms of content of the mammary gland is surprisingly low if it serves as a precursor for milk fat. The same is true of cholesterol.

The data are entirely too few to draw any other conclusions. However, attention is called to the fact that organic phosphorus content of the left gland (which milked after slaughter) is somewhat higher than that of the right half.

SUMMARY AND CONCLUSIONS

The dry matter and fat determinations of the mammary glands together with the weights of the gland tissue show that a marked atrophy of the gland takes place when the cow ceases to milk. In this atrophy

of the gland both percentage and total fat declines in spite of the fact that the animal puts on fat in other parts of the body. That the fat content of the gland decreases when the animal deposits fat in other tissues would indicate that the conditions responsible for fat deposition in the mammary gland are different than those responsible for fat deposition in other parts of the body.

The data also bring out the fact that fat is one of the chief constituents in the lactating gland, comprising, on the average, 40 per cent of the dry matter. While the data are too few to draw definite conclusions, there are indications that the ratio between the total dry matter and the fat in the lactating gland determines the fat dry matter ratio of the milk produced.

The chemical analysis of the ether extract of gland tissue brings out two important facts:

1, the nature of the fat from lactating glands differs widely from that of the non-lactating gland;

2, the fat of the lactating gland is almost intermediate in character between butterfat and body fat as judged by the three constants of fat; the iodine number the Reichert-Wollny number and the saponification number.

While the fat from the dry gland gives a small Reichert-Wollny number the saponification number and iodine number are about the same as for the adipose fat surrounding the gland.

The iodine number of the fat of the lactating gland is less than that of the adipose fat immediately surrounding the gland. The Reichert-Wollny number varies from one-fourth to one-half the value of normal butterfat, and on the average the saponification number is more than halfway between the values for body fat and normal butterfat. Why the saponification number should be higher in proportion than the Reichert-Wollny number can only be conjectured at this time. It could be explained by a less than normal amount of the intermediate fatty acids like myristic, lauric, and palmitic. This, however, remains to be proven.

The organic phosphorus and cholesterol content of two lactating glands were too low to warrant their considerations as the immediate milk fat.

It can be shown that the characteristics of the gland fat cannot be attributed to the retention of milk fat. First, the right gland was milked dry at the time of slaughter and, gauged by the record of the previous milkings, a normal amount of both milk and fat was secured. Second, based upon the saponification number of 211.1 of the gland fat more than one-half of the fat of the gland should be retained milk fat. This would in every case be over one day's normal fat production for the cow.

From these data it can be concluded that the fat in the mammary gland of lactating cows is concerned in the synthesis of the milk fat.

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THE SYNTHESIS AND SECRETION OF MILK FAT[1]

III. A Study of the Activity of the Perfused Surviving Gland, With Special Reference to the Fat

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THE PROBLEM. In the previous study it was shown that excised glands yielded the calculated amount of milk that should have been produced at the time of slaughter and that the milk was normal except for its low fat content. It has also been shown in the preceding paper that the residue from the ether extract of lactating mammary glands is intermediate in character between body fat and normal butterfat. It is therefore evident that a gland which is milked after being excised should contain the milk fat which was not secreted in the post-mortem milk and the gland fat which is more or less intermediary in character between milk fat and body fat. This experiment of perfusing surviving glands was originally planned as an endeavor to liberate these fats.

PLAN OF EXPERIMENT. In all cases the glands were removed as quickly as possible from the animal, when bled, and suspended as nearly as possible in normal position from a frame by means of wire hooks through the surrounding skin and suspensory ligament. Glass cannulas were then inserted in the external pudic arteries and connected to the container of the perfusion liquid by means of rubber tubing. The perfusion was kept at 100 to 102°F. and was elevated five feet above the gland to give approximately normal blood pressure.

The perfusion liquid was kept saturated with oxygen either by bubbling air through it or by means of a mechanical stirrer that kept the entire solution whipped up with air.

The glands from five cows were used in the study of:

1. The effect of perfusion with Locke and Rosenheim (1907) saline solution upon the amount and character of secretion of the surviving gland.

[1] The data in this paper are taken from a thesis presented to the Graduate Faculty, University of Minnesota by W. E. Petersen in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Published with the approval of the Director as Paper no. 853, Journal Series, Minnesota Experiment Station.

2. The effect of perfusion with Locke and Rosenheim saline solution emulsified with corn oil upon the amount and character of secretion of the surviving gland.

3. The effect of the corn oil emulsion upon fat deposition in the surviving gland.

4. The effect of adrenalin in reducing the edema accompanying perfusion.

5. The effect of perfusion upon the fat in the gland tissue.

No. 361 was a mature Holstein cow in milk two weeks at the time of slaughter. Each half of the udder was milked separately for three days prior to slaughter and milks were analyzed for fat and total solids. She was slaughtered at a regular milking time and only the right gland milked at slaughter time, the left gland being milked after the perfusion started. Both glands were perfused with Locke and Rosenhein saline solution and secretions expressed and analyzed. Perfusion lasted 13 hours.

No. 380 was a mature Holstein cow in milk 8 days when slaughtered, milking 30 kilos. Both glands were milked dry at slaughter time. Both glands were perfused for about 2 hours with unmodified Locke and Rosenheim saline solution; then the right gland was perfused with the saline solution in which 0.5 per cent corn oil had been emulsified. The emulsion was prepared by stabilizing with gum arabic homogenizing five times at 5,000 pounds pressure. (The left gland was continued on the unmodified Locke and Rosenheim saline solution.) Secretions from both glands were expressed separately at regular intervals and analyzed for fat and total solids.

No. 535 was a mature Guernsey cow in milk four and one-half months. The glands from this cow were treated identically to that of no. 380.

No. 408, a four year old Holstein cow in milk ten days when slaughtered was milked dry at the time of slaughter. Both glands perfused with unmodified Locke and Rosenheim saline solution. After 2 hours the right gland was perfused with a 0.5 per cent corn oil emulsion (colored with Sudan III, otherwise as previously described) for 2 hours and then changed to clear saline solution to wash out fat particles of the circulatory system. One cubic centimeter of a 1 to 10,000 solution of adrenalin hydrochloride made up according to Starling (1926) was injected hourly into the perfusion liquid going into each gland until one hour before termination of the experiment this amount was doubled for the right gland and trebled for the left gland. The fat of each gland was extracted with ether after dehydration with calcium sulphate and deposition of colored fat determined colorimetrically in a Duboscq colorimeter using a known dilution of the colored fat from the perfusion emulsion as the standard. The secretions in addition to being examined for colored fat were analyzed as in previous experiments. Sections of the gland were examined microscopically for colored fat. The

saponification number, Reichert-Wollny number, iodin number, and acid number were determined on the ether extract of the glands.

No. 158, a mature Jersey cow in milk four weeks at the time of slaughter was treated as no. 408 except Na_2HPO_4 was added to the perfusion solution so as to give 5 mgm. P per 100 cc. Also after the first injection $\frac{1}{2}$ cc. adrenalin hydrochloride solution was used hourly instead of 1 cc. as used previously.

RESULTS AND DISCUSSION. 1. *Effect of Locke and Rosenheim saline solution upon secretion of surviving gland.* From table 1 it is seen that there is

TABLE 1

Amount of milk and per cent fat and solids of successive post-mortem milkings of 361

PORTION	RIGHT GLAND			LEFT GLAND		
	Amount milk	Fat	Solids	Amount milk	Fat	Solids
	grams	*per cent*	*per cent*	*grams*	*per cent*	*per cent*
1	220	1.81	7.65	3,110	1.39	7.31
2	203	0.90	5.87	471	0.92	7.00
3	85	0.67	4.78	426	0.82	6.75
4	185	0.40	2.98	300	0.94	6.85
5	592	0.41	2.84	202	0.94	7.09
6	289	0.32	1.87	376	1.05	6.70
7	142	0.17	1.56	202	1.37	5.12
8	174	0.13	1.26	494	1.00	5.12
9	321	0.25	1.31	520	0.89	3.77
10	120	0.31	1.14	225	0.86	3.69
11	85	0.14	1.14	288	0.68	3.04
12				324	0.41	2.17
13				201	0.30	1.85
14				191	0.38	2.12
15				336	0.26	1.60
16				620	0.18	1.57
Total.....	2,416			8,286		

Right gland was milked dry at time of slaughter, producing 3,632 grams of milk with 2.82 per cent fat and 11.21 per cent dry matter.

a fairly constant diminution of both dry matter and fat in the successive post-mortem milkings. The rate of decline of the fat was relatively much greater than for the dry matter. For the left gland the higher concentration of fat and dry matter persists for a longer time than for the right gland, due to the retained milk in the left gland which was not milked before slaughter.

Table 2 gives the total fat and dry matter secreted by each gland including the pre-slaughter milking for the right gland. When so considered it is seen that the left gland secreted the same solids-not-fat but

much less fat than the right gland. The fat percentages on the basis of total dry matter are 23.97 and 17.88, respectively, for the secretions of the right and left glands.

From these data it becomes apparent that a normal amount of dry matter was secured in the post-mortem milkings of the left gland but almost 30 per cent less fat than would be expected as calculated from the production of the right gland.

It is reasonable to believe that the alveoli were filled with milk at the time milking began and that therefore a part of the dry matter secreted in the post-mortem milk came out of the alveoli. If such is the case, something prevented the secretion of a considerable portion of the fat which should have been in the alveoli. The possibility of the fat particles remaining in the alveoli due to physical retardation must be admitted. It is possible that the fat particles are too large to gain entrance to the lactiferous ducts

TABLE 2

Total dry matter and fat of the milkings from each gland of 361

	RIGHT HALF		LEFT HALF	
	Dry matter	Fat	Dry matter	Fat
	grams	*grams*	*grams*	*grams*
At slaughter	407.15	102.42	Not milked	
Post-mortem milks	71.58	12.25	451.33	80.71
Total	478.73	114.67	451.33	80.71
Solid-not-fat (grams)	263.96		370.62	
Fat on dry matter basis (per cent)	23.97		17.88	

under the post-mortem conditions. On the other hand there are some arguments against such an explanation. Microscopic examination of the post-mortem milks showed a normal distribution of large and small fat particles. If physical retardation were responsible this should not be the case as the small particles should then predominate due to the greater ease of going through the orifice of the lactiferous ducts. Another objection to the physical retardation hypothesis is the difficulty of picturing the fat particles being occluded and yet permit the fat free milk to proceed. At the temperature of the gland the fat particles are liquid and spherical. The lactiferous ducts are more or less cylindrical. Under these conditions it would appear that the passage of milk would be prevented should the fat particles be retarded due to their size.

It is therefore necessary to consider some other explanation for the failure to account for all the fat in the post-mortem milkings. It is suggested that some altered condition in the alveoli prevents the liberation of the fat. That the conditions in the alveoli are altered in perfusion with

saline solution finds support in the character of milks secured during the later parts of perfusion tests. In all the perfusion milks after the first 2 or 3 hours the proteins were precipitated into a heavy flocculent material that rapidly settled to the bottom. Titration with NaOH to the alkaline side of neutrality failed to bring the precipitated material back into dispersion. It is conceivable that some similar effect might be had upon the cell protoplasm which in turn would prevent the liberation of the fat.

2. *Effect of emulsified corn oil upon secretion.* The results from perfusing four glands with an emulsion of Locke and Rosenheim solution with 0.5 per cent corn oil failed to show any increase in fat percentage. Table 3 giving the analysis of the successive post-mortem milkings of cow 535 is typical of the other three. The milks from the right gland have approxi-

TABLE 3

Fat and dry matter of post-mortem milkings of 535

MILKING	RIGHT GLAND			LEFT GLAND		
	Amount milk	Fat	Dry matter	Amount milk	Fat	Dry matter
	grams	*per cent*	*per cent*	*grams*	*per cent*	*per cent*
10:00 a.m.	41	4.7	12.8	51	3.9	12.95
11:00 a.m.	57	0.65	7.13	62	0.6	6.84
12:00 m.	100	0.55	4.79	92	0.6	4.88
1:00 p.m.	213	0.30	2.34	193	0.45	3.02
2:30 p.m.*	255	0.12	1.56	307	0.35	2.90
3:00 p.m.	218	0.08	1.26	221	0.12	1.56
4:00 p.m.	179	0.09	1.22	217	0.15	1.32

* Right gland had been perfused with emulsion for one-half hour. This was continued to end. Glucose added to perfusion liquid of both halves.

mately the same fat percentage as those of the left gland which were not perfused with the oil emulsion. Further proof that the fat of the emulsion did not pass into the secretion is offered with the experiments on cows 408 and 158 where the oil was colored with Sudan III. Careful examination failed to reveal any colored fat particles in the milks. This does not confirm Foa's (1911) work when he reported perfusing sheep's glands with an emulsion of tri-olein which resulted in an increase in the fat content of the milk.

3. *Effect of emulsified corn oil upon fat deposition in glands.* The glands perfused with the emulsion of corn oil stained with Sudan III were a uniform pink throughout. Microscopical examination of sections revealed only an occasional colored fat particle in capillaries, while the fat cells seemed to be fairly highly colored. The ether extract of the dehydrated gland tissue, perfused with the colored emulsion, was highly colored. On the basis of colorimetric comparisons it is calculated that for cow 408, 2.17

per cent of the total fat of the gland was deposited from the perfusion liquid while for cow no. 158 this figure was 3.21 per cent.

From this it would appear that the finely emulsified fat of the perfusion liquid was deposited in the fat cells of the gland, and in large enough quantities to take care of any fat metabolism. This is contrary to common belief as it is generally held that fats permeate the cell wall chiefly in the form of phospholipoid. Bloor (1922) takes the stand that most of the stored fat in the tissues comes from phosphatides although some fat may be deposited directly from the neutral fat of the blood stream. Again the work of Fish and Gage (1924) in demonstrating the increase of chylomicrons in the blood following a fatty meal raises the question of the necessity of phosphatides for transport of fat to the cell.

4. *Effect of adrenalin upon reduction of edema.* In all perfusion experiments with salt solutions edema of the gland developed to a marked ex-

TABLE 4

Analysis of the ether extract of perfused mammary glands

FAT CONSTANT	ETHER EXTRACT	
	Gland 408	Gland 158
Reichert-Wollny number	27.35	23.80
Saponification number	210.77	208.35
Iodine number	38.42	43.01
Acid number	8.07	11.30

tent. In dissection of some of these glands it was noted that the arteries had become dilated and the interstitial tissue filled with fluid. It appeared as though the vessel walls had lost their tonicity and that such might be prevented by using adrenalin in the perfusion liquid. With the gland of no. 408 1 cc. adrenalin hydrochloride solution hourly reduced the edema markedly and caused the vessel walls to retain their tonicity until the end of the experiment. In the second case the same dosage proved too large as almost immediately after the injection of the solution the capillaries closed up so as to scarcely permit the flow of the perfusion liquid. Increased dosage at the close of the experiment showed that the glands were responsive to adrenalin 6 to 8 hours after the beginning of perfusion.

5. *The effect of perfusion upon the character of the fat in the gland.* In only two of the perfused glands has sufficient fat been extracted to determine the fat constants. The results are given in table 4 and are given as preliminary data. It is to be noted that the Reichert-Wollny number in both cases falls in the range of normal butterfat. The saponification number is low and the iodin number is a little high. It appears that per-

fusion has changed the fat of the gland toward that of normal butterfat in so far as the volatile soluble fatty acids are concerned.

SUMMARY OF THE PERFUSION EXPERIMENTS

Five experiments involving five glands are reported in the study of perfusing with an isotonic saline solution with various modifications.

Perfusion failed to cause secretion of milk fat calculated to be in the gland. In all cases the secretions resulting from perfusion had an extremely low fat content. The fat percentage of the total solids decreased as the milking progressed. While the fat content of the post-mortem milkings was small, microscopic examination revealed a normal distribution of large and small fat globules. This, together with failure to find free fat particles in the alveoli is taken as evidence against the hypothesis that the increase in fat with the progress of milking is due to physical retardation of the fat particles by smallness of the ducts.

Perfusing corn oil emulsion through four glands failed to cause an increase in the fat content of the resulting secretion. In two cases the oil of the emulsion was stained with Sudan III and the fat of the secretion was found to be free from color. It is therefore apparent that the fat of the circulating fluid does not pass directly into the secretion.

The emulsified fat of the perfusion liquid apparently is capable of being deposited in the fat cells of the gland. Microscopic sections of glands perfused with the colored emulsion showed fairly uniform coloration of the fat cells. The extracted fats of the glands were compared, colorimetrically, with the stained fat of the emulsion from which it is estimated that 2.17 per cent and 3.21 per cent respectively of the fat of two glands had originated from the perfused fat emulsion.

Chemical analysis of the extracted fat from two glands showed that the volatile fatty acids as shown by the Reichert-Wollny number were increased by perfusion to that of practically normal butterfat. The saponification number and iodine number, however, were not affected. The results are interesting in that it is evident some change is brought about in the ether soluble material. The exact significance can be ascertained only through further investigation. It may be pointed out, however, that to get an increase in the Reichert-Wollny number without an increase in the saponification number there must be present either volatile acid of high molecular weight or a non-acid compound that is soluble in fat solvents and which is converted into volatile acids by the sulphuric acid hydrolysis of the Reichert-Wollny process.

It has been shown that addition of adrenalin to the perfusion liquid was responsible for an extended retention of the tonicity of the blood

vessels. The amount necessary apparently differs, as what was found to be right in one case was too much in another.

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THE TRANSPARENCY OF LIVE AND DEAD ANIMAL TISSUE TO ULTRA-VIOLET LIGHT[1]

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The penetration of light through animal tissue is usually tried on dead material. The literature of the most important investigations about this subject, with particular attention to the skin, is given in the publication of Bachem and Kunz "The transmission of ultra-violet light through the human skin" (1). Recently however Macht and his co-workers (2) (3) have published the results of extensive investigations on living skin. The principal results are given by these authors as follows:

(2) "It was definitely established that penetration of ultra-violet rays through the living skin and other tissue is much greater than has hitherto been supposed.

(3) "Some of the shorter ultra-violet rays penetrate through the living skin more deeply than the longer ultra-violet rays.

(4) "A marked difference was noted between the living skin and the dead skin."

The methods used and the conclusions arrived at by these authors were criticized by several authors (4) (5) (6). This induced Anderson and Macht to repeat their measurements with much improved methods.

In a recent publication (7) they admit certain errors in their former results; they find a uniform transmission of 6 to 10 per cent through the whole ultra-violet for 1.2 mm. living rabbit skin, instead of from 11.4 per cent at 3660 AU to 56.3 per cent at 2800 AU and 42.8 per cent at 2537 AU. Even with this correction the results seemed doubtful, when compared with the results of many other investigators and those obtained by Bachem and Kunz (1) with dead tissue. As to the comparison of live and dead tissue, their experiments do not agree, either, with another experiment of Bachem and Kunz, in which the ears of rabbits, one cut off 2 days prior and left in Ringer solution, the other left alive, did not show any measurable difference. Therefore, we decided to investigate systematically the possible difference between live and dead skin.

[1] A part of the expenses of this investigation was paid from a grant from Phi Rho Sigma Medical Fraternity.

For these experiments we used dogs first and later young rabbits. The skin of the abdomen and the inner skin of the ear was prepared while the animal was anesthetized by ether or barbital, and held tight against the slit of a Hilger quartz spectrograph. After an exposure of 400 seconds to the Kromayer lamp the skin was cut off and either left in exactly the same position, care being taken that it could not shrink and bend; or the skin was cut off and stretched over a cork with a central hole, kept tense by rubber bands and put in Ringer solution. From time to time these specimens were put before the slit, exposed for the same period and returned to the solution for later experiments. Several comparison spectra were made with 1/25, 5/25, 1, and more seconds, in order to get quantitative results for the transmission, with the Schwarzschild factor established beforehand, which allows an intensity comparison by a comparison of exposure times,

TABLE 1

Percentage penetration

λ	LIVE	½ HOUR DEAD, DRYING OUT	20 HOURS DEAD, DRIED OUT
	per cent	*per cent*	*per cent*
578	0.1	0.3	1.7
546	0.15	0.5	2
435	Trace	Trace	0.3
401	—	—	—
365	Trace	Trace	0.2
334	—	—	0.1
313	—	—	0.05
302	—	—	—

Skin, dog abdomen, 0.93 mm. thick.

The exposures for live and dead tissue and the comparison spectra were made on the same photographic plate. This prevented errors on account of irregularities in the mode of developing the plates, and it gave the most convincing results, as to an increased or lowered penetration.

The direct results are given in tables 1 and 2.

These tables reveal that drying out increases the transparency of the tissue. As soon as the skin looks like parchment, it transmits much more light, in the ultra-violet as well as in the visible part of the spectrum. In the next tests two pieces of live skin 0.27 mm. thick, from the abdomen of a rabbit, were observed, one of them being permitted to dry out, the other one kept wet in Ringer solution after having been cut off. The transmission through the live skin was about 0.5 per cent from 578 to 491$\mu\mu$; it was much smaller at 405$\mu\mu$ and fell to zero at about 300$\mu\mu$. The transmission of the drying specimen increased rapidly for the time of drying out, par-

ticularly between ½ and about 2 hours after being cut off, reached a maximum after about 3 hours, after which it declined very gradually. The transparency maximum corresponds to a minimum of scattering; at this time the skin has the appearance of parchment; the colloidal material has changed into a more homogeneous solid body, in which true absorption is the determining factor, besides a remnant of absorption due to scattering. The later increase of absorption may be explained by the appearance of small cracks and air enclosures.

The specimen kept in Ringer solution did not show such marked changes. The penetration remained nearly constant for the first hour, after which it declined for a few hours, to become more constant again. An exception

TABLE 2

Percentage penetration

λ	LIVE	15 MINUTES DEAD, DRYING OUT	12½ HOURS DEAD, DRY
	per cent	*per cent*	*per cent*
578	0.5	0.5	0.5
546	0.7	0.7	0.5
435	0.3	0.3	2.0
401	0.05	0.05	0.9
365	0.2	0.12	2.0
334	Trace	Trace	1.2
313	0.01	0.01	1.1
302	0	0	1.1
297	0	0	0.5
293	0	0	0.1
289	0	0	0.06
285–257	0	0	0
253	0	0	Trace
248	0	0	0

Skin, rabbit abdomen, 0.20 mm. thick.

to this rule was evident at 405$\mu\mu$, where the hemoglobin absorption is very pronounced. A gradual increase of the transparency indicated that hemoglobin was washed out by this kind of preservation. All together there was very little change in transparency, in disagreement with the claims of Macht and his co-workers. There was a certain change however in the appearance of the spectral lines, those of live skin showing much "detail" due to capillaries, hair follicles, and other irregularities, those of the dry and wet specimens showing a foggy or even homogeneous structure.

In order to secure higher penetration and to extend our observations into the far ultra-violet, we prepared the inner skin of two rabbit ears for observation, first alive, then one dried and one kept wet. This skin was 0.12 mm. thick.

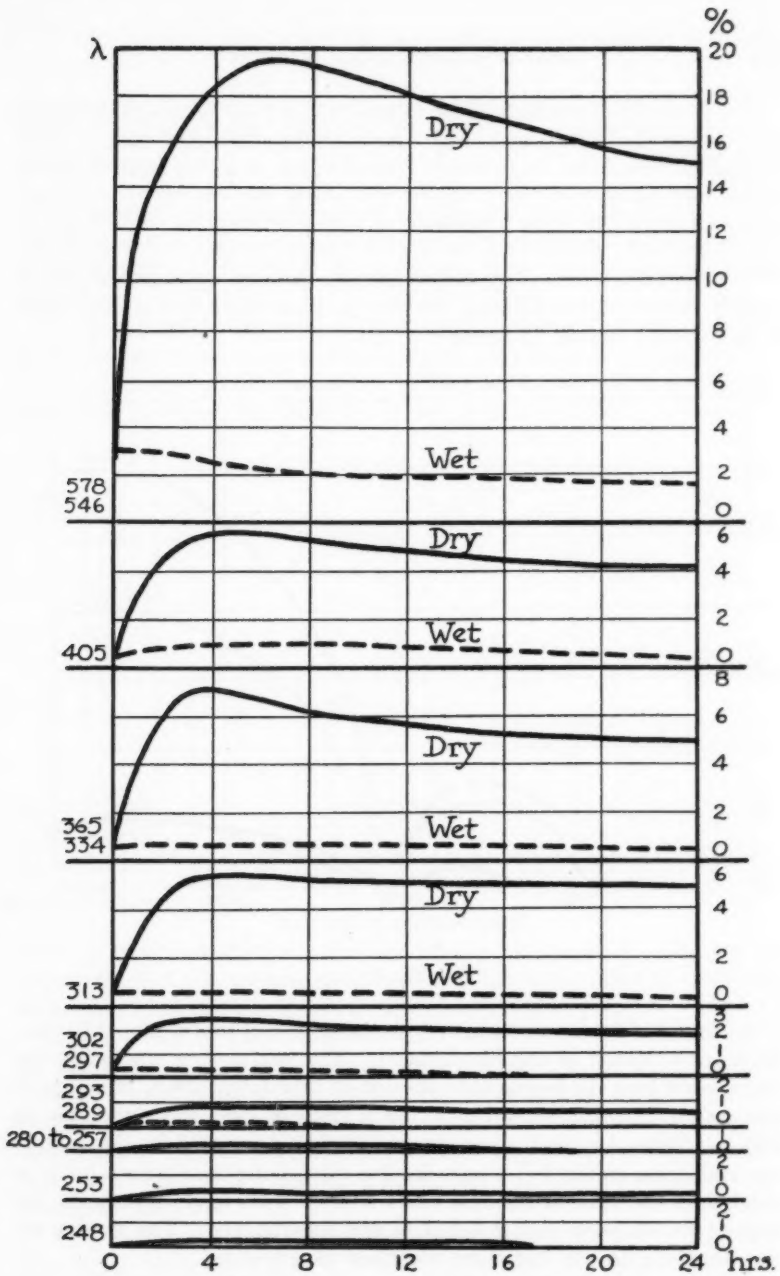
λ
%
Dry
Wet
578
546
405
365
334
313
302
297
293
289
280 to 257
253
248
0
4
8
12
16
20
24
hrs.
Skin, rabbit ears, .10 mm. thick; dry, wet

Fig. 1

The penetration reached higher figures, up to 2 per cent and extended farther into the ultra-violet, with traces at 293, 289, and 253$\mu\mu$. The hemoglobin minimum was more pronounced, due to a thin layer of blood, which was intentionally left, in order to study the effects. In the dry specimen it prevented the transparency from increasing; in the wet specimen it gradually disappeared and caused a sharp increase, so reversing the effects approximately. The maximum for the dry specimen appeared usually between 2 and 4 hours, the wet specimen was very constant, with a mild decline toward 55 hours.

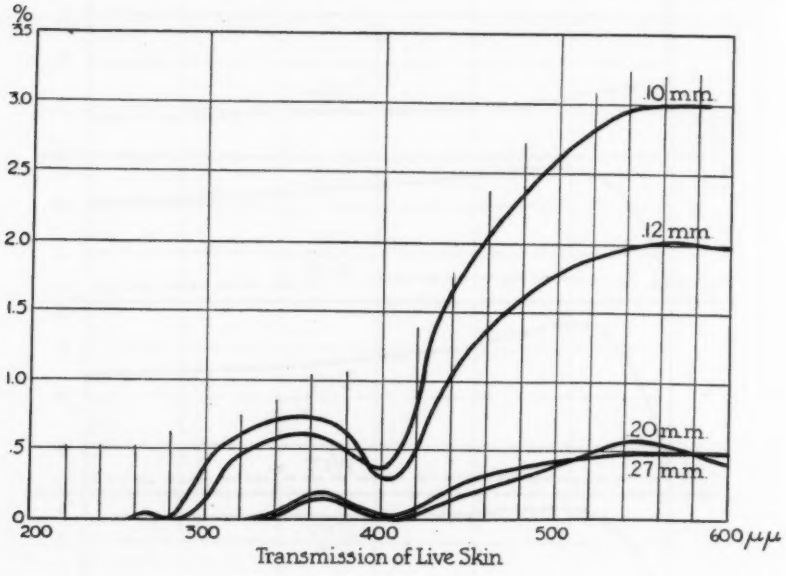

Fig. 2

A last experiment was made with the inner skin of rabbit ears, 0.10 mm. thick, well cleaned of any blood stains. The results were practically the same; even here the hemoglobin absorption manifested itself, pronounced absorption occurred below 289$\mu\mu$, but a trace of light was noticeable at 253$\mu\mu$. Figure 1 gives the percentage of light transmitted for the various spectral lines, at the left corner for live skin; along the abscissa for skin dead from the time of removal to 24 hours. This figure represents the highest transmission ever reached in our experiments. The results are characteristic for the last three described experiments.

In this experiment a part of the wet specimen was compressed for a few

seconds after 23 hours; this resulted in a pronounced increase of the transmission, indicating that by squeezing out water a more homogeneous structure was obtained. The transmission curves for live skin are plotted in figure 2. The figures differ strongly from those obtained by Macht. They prove that the transparency generally decreases from visible to near, and from near to far ultra-violet, with the two selective absorption bands of the hematin at 400 and of the globin at 280$\mu\mu$. No transparency was found to exceed 0.1 per cent in the far ultra-violet.

The pronounced difference of transparency of dry and wet specimens gives us a chance to estimate and compare the effects of pure absorption and of absorption due to scattering. The maximum transparency of dry specimens is determined mainly by the true absorption coefficient $\bar{\mu}$,

TABLE 3

SPECIMEN	0.27 MM., ABDOMEN					0.12 MM., EARS					0.10 MM., EARS				
	Per cent					Per cent					Per cent				
λ	Maximum dry	Corresponding wet	$\bar{\mu}+$	$\mu-$	$\sigma-$	Maximum dry	Corresponding wet	$\bar{\mu}+$	$\mu-$	$\sigma-$	Maximum dry	Corresponding wet	$\bar{\mu}+$	$\mu-$	$\sigma-$
546; 578	4.0	0.3	5.19	9.30	(4.11)	10	2	8.33	14.20	(5.87)	20	2	7.0	17.0	(10.0)
492	2.1	0.2	6.21	10.00	(3.79)	6	1.2	10.2	16.0	(5.8)					
435	1.6	0.2	6.68	10.00	(3.32)	2	0.8	14.2	17.5	(3.3)					
405	1.0	0.1	7.4	11.5	4.1	0.2	0.8	22.5	17.5	—	5.4	1	12.7	20.0	(7.3)
365	1.3	0.1	7.0	11.5	4.5	1	0.7	16.7	18.0	(1.3)	7	0.6	11.6	22.2	10.6
334	1	0.05	7.4	12.2	4.8	2	0.5	14.2	19.2	5.0					
313						1.5	0.4	15.2	20.0	4.8	5.4	0.4	12.7	24.0	11.3
297; 302						1	0.35	16.7	21.7	5.0	2.4	0.2	16.2	27.0	10.8
289; 293						0.5	0.1	19.2	25.1	5.9	0.7	0.05	21.6	33.0	11.4
265; 280						0.1	0.03	25.0	29.4	4.4					
248; 253						0.2	0.04	22.5	28.4	5.9					

True absorption and scattering.

and to a small extent only by the coefficient of scattering σ. The smaller transparency of the wet specimens is caused by a larger effect of scattering absorption in addition to the same true absorption, $\mu + \sigma$.

In case of a very narrow beam of light observed exactly in direction of the incident radiation (without any stray radiation) it would be the total absorption coefficient μ. In case of the spectrograph work it will not exactly reach that theoretical value. The difference between the two coefficients μ + small part of σ and μ + very large part σ will give us a minimum value for the scattering coefficient σ. This calculation is performed in table 3.

These figures indicate that the increased absorption from the visible toward the ultra-violet part is mostly due to true absorption; the scattering seems to be nearly constant over the investigated range. Of course

the scattering is greater than $\sigma -$ in every last column; how much, cannot be determined by this experiment. Therefore these figures are to be considered as preliminary and as an attempt to untangle the two kinds of absorption. It seems that for visible light the scattering predominates; for ultra-violet however the scattering is far exceeded by true absorption. This problem of true absorption and scattering will be investigated by us in the near future.

SUMMARY

1. It can be shown by an extremely simple and conclusive method that little difference of transmission exists between live and dead tissue, for the next few hours after death, if kept wet in Ringer solution and well stretched. Therefore with proper precautions dead tissue can be used for the study of light transmission through animal skin.

2. The pronounced difference of transmission through dried and wet skin permitted estimation of the relative importance of true absorption and scattering. The true absorption coefficient was found to change strongly with the wave length, the scattering coefficient was found to be nearly constant.

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STUDIES ON THE INTESTINAL MUSCLE OF MAN

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Much is known about the rhythmic contractions of the excised bowel of animals but little has as yet been learned about this type of activity in the bowel of man. It seemed to us worth while, therefore, to put on record some observations made on bits of intestinal muscle obtained during the course of thirteen necropsies. The interval after death varied from one and a half to six hours. Short segments of duodenum, jejunum, upper ileum, lower ileum, and ascending colon were removed; they were washed and kept in cold Gasser's solution until the laboratory was reached. Small strips 8 mm. wide and 25 mm. long were then cut with the longer side following the longitudinal axis of the bowel. The mucous membrane was left adherent so as to avoid further injury to the muscle. These small pieces were placed in warm aërated Gasser's solution and fastened with the help of small wire serrefines and threads to light levers writing on a smoked drum.

Rhythmic contractions could be induced in only four of the sets of strips studied. The most active ones were obtained from a man who, two hours before necropsy, died of acute nephritis. Within a few minutes after transference of the segments to the beaker of warm aërated saline solution the duodenum began to contract with a slow tonus rhythm. Three minutes later the jejunum and upper ileum began to contract and a minute later the lower ileum contracted slightly. Thirty minutes later this last segment began to show regular rhythmic waves, and at the same time the colon began to contract with a series of tonus waves similar to those seen in the large bowel of laboratory animals (fig. 1). The only part of the bowel in which tonus changes were slight was in the upper ileum.

As one of us (Alvarez) has pointed out, the rhythmic contractions in man are seldom regular like those of the rabbit but are usually irregular and much like those of the dog and cat. The rate changes suddenly from minute to minute, and there is no constant gradient in rate of rhythmic contraction from duodenum to lower ileum (table 1). As one would expect also from the work on animals, the rates of contraction in these excised bits of muscle are slower than those noted in living men and women.

After these studies had been made, the segments were put in cold Gasser's solution and left overnight in the ice box at a temperature of from

5 to 10°C. Thirty-six hours after removal from the body they were again placed in warm aërated salt solution and again the duodenum began to contract first, the jejunum second, the upper ileum third, and the terminal ileum fourth. The piece from the colon did not recover its ability to contract rhythmically.

The duodenum contracted as well as it did on the first day, but after a few beats the other strips showed signs of fatigue and the amplitude of contraction diminished. Tonus waves were prominent in the duodenal record but they soon disappeared from those of the other segments. As will be seen from table 1, there was at times a gradation in the rate of rhythmic contraction from duodenum to ileum.

TABLE 1

Contractions in each minute

	FIRST CASE		SECOND CASE	THIRD CASE	FOURTH CASE
	First day	36 hours later			
Duodenum	5.0, 7.0, 8.0, 7.0 8.0, 6.0, 6.0, 6.0	7.0, 7.0, 6.0 7.0, 6.0, 7.0 Tonus waves	4.5, 4.7 5.3, 7.5, 8.0	One every 3 minutes	Tonus waves irregular
Jejunum	4.5, 4.5, 4.0 4.0, 4.0, 4.5	6.0, 5.0, 5.0	2.0, 2.3, 2.0 3.0, 2.3, 4.0, 5.3		Tonus waves irregular
Upper ileum	8.5, 8.0, 7.0 Tonus waves	4.5, 4.0, 5.0			1.5, 1.5 1.5, 3.0
Lower ileum	Tonus waves 5.0 2.0	7.0, 9.0, 9.0 3.5, 3.5			1.5, 1.0 9.0?, 9.0?
Colon	Small tonus waves	Tonus waves		1.0, 2.0	1.5, 2.0 2.5

The second set of strips was obtained from the intestine of a man who died shortly after swallowing hydrochloric acid. Necropsy was performed one and one-half hours after death. The duodenum contracted first and the jejunum second; later the other two strips showed slight rhythmic changes in tone. As will be seen from figure 1, the small contractions were irregular and the rate was slow. After the strips had been in the ice box for twelve hours the duodenum and jejunum contracted again just as they did on the first day and at practically the same rates. They responded well to stimulation with eserin.

The third set of strips was obtained from the intestine of a girl who died in diabetic coma. Necropsy was performed one hour and forty-five minutes after death. Ten minutes after the strips were placed in warm Gasser's solution, there was a sharp rise and fall in the duodenal record and

later such "humps" appeared at long intervals. The strip from the upper ileum showed slight rhythmic contractions and the colon showed rhythmic contractions superimposed on slow tonus waves. At no time did the other two strips contract rhythmically.

The fourth set of strips was obtained from the intestine of an infant boy who was born dead after a difficult breech delivery. A few minutes later some ice water was injected into the stomach with the idea of cooling the abdominal contents. The body was kept in a cold room for four hours until permission was obtained for the necropsy. The two strips from upper and lower ileum were the first to contract and the colon followed four minutes later with slow tonus waves. Later these waves disappeared

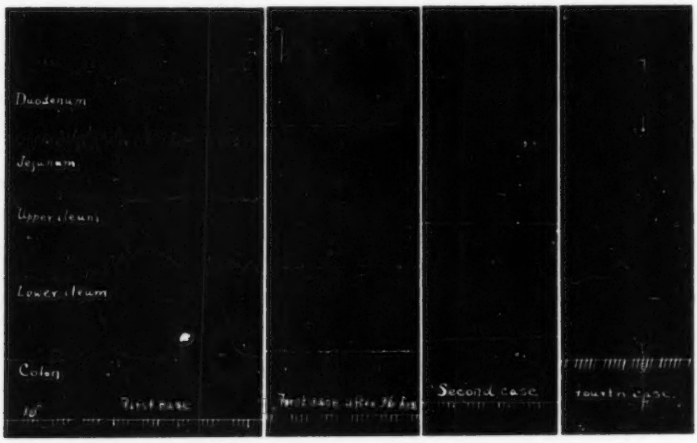

Fig. 1. Records of the activity in segments removed from the bowel of man

and rhythmic contractions became fairly rapid and regular. The duodenum and jejunum showed tonus waves but very few of the small rhythmic contractions. The lower ileum contracted with a rapid, regular rhythm similar to that seen in the intestine of the rabbit. The other segments contracted slowly and irregularly. There was no gradient (from duodenum to ileum) in the rate of rhythmic contraction.

Contractions could not be induced in any of the other nine sets of strips. In six of them this was probably due to the fact that the bits of bowel were not removed until more than two hours had elapsed after death. In two cases it is possible also that the peritonitis which produced death injured the muscle. We were surprised at our failure to evoke contractions in muscle removed two hours after death from a man who succumbed to

acute nephritis because the conditions were so similar to those in the first case in which good records were obtained.

The observations here reported agree with those of Alvarez in that a definite gradient in rate of rhythmic contraction was not found. It is suggestive, however, that just as in the rabbit, so in man, there appears often to be a gradient in the tendency to contract rhythmically; that is, the waves appear first in the duodenum and then at successive intervals in the jejunum, lower ileum, upper ileum, and colon.

Our results in these cases and our experience with segments of bowel removed from animals at varying intervals after death led us to believe that the absence of rhythmic contractions in most of the strips could be accounted for by the long interval that elapsed between the stoppage of the circulation and the removal of the bowel from the warm body. In order to learn a little more about the conditions that favor or hinder the death of intestinal muscle we performed on rabbits a series of experiments which will be described in a subsequent paper.

SUMMARY

A study has been made of the rhythmic contractions which appeared in excised strips of human bowel. The rates were slower than those observed in living men and women; they varied from time to time, and there was little sign of a gradient from duodenum to ileum. There were signs, however, of a gradient in the tendency to contract rhythmically, with the duodenum first and the colon last.

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FACTORS THAT INFLUENCE THE CONSERVATION OF INTESTINAL RHYTHMICITY AFTER DEATH

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The study here reported was undertaken with the hope of throwing light on the factors which, after the death of an animal or a man, bring about a loss of the ability of the bowel to contract rhythmically. As will be seen in figure 1, the death of a rabbit causes first aslowing and soon a cessation of the rhythmic movements. If segments of intestine from such an animal are then cut out and transferred to warm aërated Locke's solution they will again contract rhythmically and they will continue to do so for six hours or more. If they are cut out and kept in iced Locke's solution they will, when transferred to warm oxygenated Locke's solution, contract rhythmically as late sometimes as the fifth day after excision.

What has puzzled us is the immediate slowing and often rapid cessation of movement which is observed when the bowel is left in situ after death. If segments excised and placed in a beaker of warm aërated Locke's solution can contract rhythmically for hours, why should they not do the same when left attached to the mesentery and with the abdomen opened in a large tank of aërated Locke's solution? Is the difference to be accounted for by the connection of the bowel with the animal or by the assumption that there is less oxygen in the larger amount of fluid? The latter explanation does not seem tenable because Alvarez and Starkweather showed years ago that the amount of oxygen normally present in unboiled Locke's solution is enough to keep segments of lieum beating well for some time.

That the retention of the nerve supply of the bowel might have some influence was suggested by some work of von Uexkull on lower forms of life which led him to believe that if at the time of death a contractile organ is left with its nerve supply intact something which would otherwise remain trapped in the tissue, to maintain tone, withdraws into the ganglion cells. Such a theory can hardly be used to explain our observations on the dying bowel because the phenomena were so little changed after degenerative section of the vagi and major splanchnics (fig. 2).

It will be noted that after degenerative section of the splanchnics, or of the vagi and splanchnics together, death did not produce the sudden

slowing of the rate. This appeared to be due to the fact that the slowing had already taken place, and it suggests that the slowing in normal animals is due not so much to the loss of the circulation as to the cessation of function in some part of the enteric nervous system. Against this explanation is the fact that the slowing does not take place immediately after section of the nerves but comes later when they have degenerated. It seems

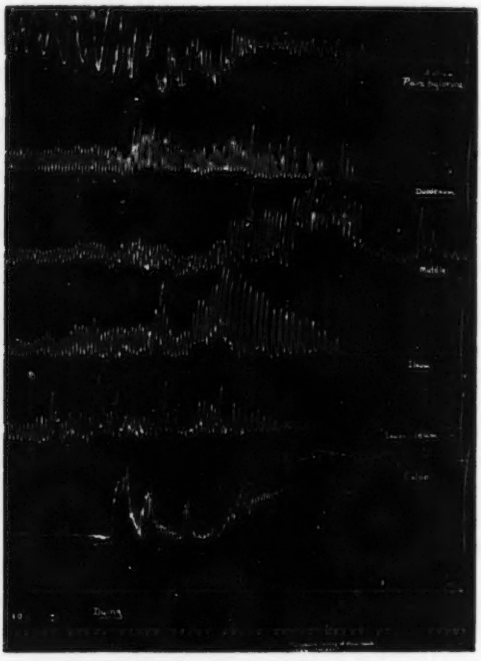

Fig. 1. Slowing and cessation of rhythmic movement in the stomach and bowel of the rabbit at the moment of death.

improbable also from what we know of the survival of nerves that their function should cease within a few seconds after the death of the animal.

Another explanation is that at the moment of death the preganglionic neurones, which affect the intestinal muscle only through synapses with other intermediate nerves, so lose their function that the postganglionic inhibiting fibers, which run directly to the muscle, can dominate the situation (until the bowel is removed from the body). Much against this idea is the fact that the synapses in the autonomic nervous system do not appear to lose their function until some time after the death of the

animal (Danilewsky). Obviously the problem is far from solved. More information in regard to it will be found in the discussion of a paper soon to appear on the effects on the digestive tract of degenerative section of the vagi and the splanchnics (Alvarez et al., 1929).

FACTORS THAT RETARD THE DEATH OF THE EXCISED BOWEL. In order to throw light on the factors that hasten or retard the death of the bowel we made a series of experiments on rabbits. First, four animals were killed, two by bleeding and two by a blow on the head. The bodies were kept at room temperature for one hour and segments were then

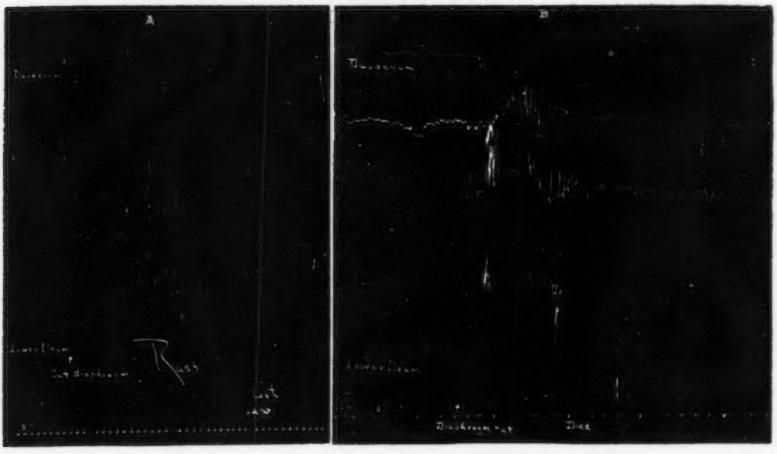

Fig. 2. *a*, Cessation of rhythmic activity in the small bowel of a rabbit in which thirty days before both splanchnic nerves had been cut and allowed to degenerate: *b*, changes in the rhythmic contractions of the small bowel of a rabbit in which thirty-one days before both vagi and both splanchnic nerves had been cut.

removed and suspended in warm oxygenated Gasser's solution. None of these segments contracted rhythmically, so evidently one hour at temperatures ranging from 38° to 21°C. is enough seriously to injure the neuromuscular apparatus.

Another animal was killed by a blow on the head. The abdomen was immediately opened and the body was placed in a tank of Gasser's solution at 21°C. After one hour segments were removed and studied in the usual way in a beaker of warm aërated Gasser's solution at 37°C. All of them contracted normally except the one from the colon which was irregular in its activities. This experiment showed that the more rapid reduction of the temperature from that of the body to that of the room made a

great difference. It is possible also that toxins formed in the bowel were able to diffuse into the surrounding solution.

Nine rabbits were next killed by bleeding. That the Gasser's solution being used was properly made was shown by the fact that segments removed immediately from one of the animals contracted normally. Four of the bodies were kept in the ice box at a temperature slightly below freezing: one for one hour and forty-five minutes, one for one hour and fifty minutes, one for two hours, and one for three hours and twenty-five minutes. Segments removed from the first animal contracted poorly but those from the other three did not contract at all. Apparently, then, the body of a small furry animal such as a rabbit is not cooled rapidly enough even when put into an ice box.

In the last four of the animals killed by bleeding, the peritoneal cavity was immediately filled with iced Gasser's solution injected through a trocar. This was done so that cooling would take place more rapidly. The bodies were then left in the ice box, one for three hours, one for three hours and fifty minutes, and two for nineteen hours. In one of those kept for nineteen hours the peritoneal cavity was washed out twice with the cold salt solution. The segments removed from the first two and studied in the usual way showed good rhythmic activity. Those removed after nineteen hours did not do so well, but fair results were obtained with segments from the animal in which the cooling solution was injected twice.

In another set of experiments the animals were killed by a blow on the head and the blood was left in the vessels. Iced Gasser's solution was injected into the peritoneal cavity and the bodies were kept in the ice box, two for two and a half hours, one for three hours, two for twenty hours and one for twenty-four hours. Segments removed after two and a half hours contracted normally. Those removed after three hours showed some diminution in rhythmicity, and the rates were slower than normal. One set of segments removed after twenty hours contracted well whereas the other set did not contract at all. The first set came from the animal whose peritoneal cavity had been washed twice. The segments removed after twenty-four hours did not contract rhythmically in spite of the fact that in this animal the solution had twice been injected.

It appeared from these experiments that the presence of blood in the intestinal vessels helped somewhat in maintaining the rhythmicity of the segments. The importance of rapid cooling was again demonstrated.

In one experiment actively contracting segments removed two hours after death were left attached to the levers for ten hours. Three times during the first six hours the solution was changed and each time the diminished amplitude of the contractions was restored to normal. We then left the laboratory and when we returned four hours later contractions had ceased, and the changing of the solution no longer brought with it revival of the

segments. From this experiment it appears that a bowel which stops contracting if left for a few minutes in the abdomen at body temperature will remain active for hours if kept at the same temperature in a beaker of aërated saline solution. The important factor is probably that of oxidation; when this is deficient the bowel must immediately be cooled or it will die.

Similar observations have been made by Nolf while working with excised segments of the thick-walled bowel of the chicken. He found that he had to keep the muscle well supplied with oxygen except at those times when metabolism was stopped by rapid chilling of the tissue. Immediately after the death of the animal the gut had to be removed and transferred to iced Ringer's solution. This had to be kept well oxygenated until the tissue reached the temperature (about 0°C.) of the solution. Then and only then could the stream of oxygen be stopped. The best results were obtained when washed red blood corpuscles, well filled with oxygen, were added to the cold Locke's solution.

But to return to the discussion of our experiments: two rabbits were killed and immediately after death segments were removed and placed in cold Gasser's solution. They were left for twenty-four hours in the ice box. When transferred to warm Gasser's solution and supplied with oxygen they contracted fairly well, which showed again that prompt cooling in the absence of aëration will prevent the autolytic changes that lead to death. Actually, as one of us (Alvarez) has shown with the thick-walled bowel of the cat and dog and man, when the infected and rapidly autolyzable mucous membrane is removed, the rhythmic contractions of the muscle are often better on the second or third day of refrigeration than they are immediately after excision of the bowel.

In all the experiments in which the segments were somewhat injured the behavior of the colon was poor and erratic. In most cases the duodenum and jejunum seemed to survive best whereas the lower ileum and colon suffered most. Often when segments from the upper part of the bowel were contracting regularly the contractions of the lower part of the ileum were so irregular that they could not be counted. These observations agree with many others made by one of us (Alvarez) all showing that there is a greater tendency to rhythmic contraction in the orad part of the bowel. The long survival of segments from the upper part of the bowel calls to mind the fact that with surviving bits of heart muscle, those from the region of the auricular sinus are generally the last to die.

The observations here reported suggest strongly that satisfactory studies of human intestinal muscle could be made if, during the interval between death and the necropsy the physiologist, intent on securing bits of viable muscle, could wash the stomach and colon repeatedly with iced saline solution.

SUMMARY

In the dying animal the rhythmic contractions of the small bowel are slowed and are usually soon brought to a stop. They become active again when the bowel is cut out and suspended in warm oxygenated Locke's solution.

After degenerative section of the vagi, of the major splanchnics, or of both sets of nerves, death produces little if any slowing of the rhythmic contractions because they have already been slowed by the destruction of the nerves. The rhythmic contractions are soon stopped but not so quickly as in normal animals.

When an interval must elapse between the death of an animal and the use of excised segments of bowel it is essential that the animal or the segments be cooled immediately. If the tissues are to survive for a few hours or days metabolism must go on in the presence of sufficient oxygen or it must not go on at all.

In order to cool the bowel rapidly one must either remove it immediately after death and place it in iced Locke's solution or else one must repeatedly wash the peritoneal cavity with the cold salt solution and at the same time put the body into an ice box.

The fact that contracting segments of excised intestine can be revived repeatedly by the changing of the solution bathing them shows that toxic or inhibiting substances are formed and excreted.

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MECHANISM OF OVULATION IN THE RABBIT

II. Ovulation Produced by the Injection of Urine from Pregnant Women

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From the demonstration that ovulation may occur in the transplanted ovary of a rabbit (1), it is obvious that the ovarian nerves are not indispensable to the process, and it is equally obvious that there is present some humoral mechanism. The question arises, however, of the significance of this humoral mechanism when the nerve supply to the ovary is intact. If it is of great significance, one should be able to provoke ovulation in the unmated rabbit by reproducing those humoral changes which normally follow coitus, and which were presumably responsible for the occurrence of ovulation in the transplanted ovary.

Experimental methods and results. A. *The transplantation of fresh rat hypophyses.* From the recent work of Smith and Engle (2), it appears that in the rat, at least, the ovulatory mechanism is stimulated by some substance, or substances, in the anterior lobe of the hypophysis. Naturally, there came the suggestion that these substances might stimulate ovulation in the rabbit also. Consequently, four non-pregnant female rabbits were subjected to hypophyseal transplantation. Following exactly the technique of Smith and Engle, five fresh rat glands were transplanted into each of the four does on each of three successive days. Six days after the first transplantation, the rabbits were killed and autopsied. The ovaries of each animal contained a normal number of ripe follicles, but no corpora hemorrhagica and no corpora lutea.

Subsequently, two non-pregnant females received at one intraperitoneal injection twenty-four fresh rat glands macerated in a small amount of sterile saline. At autopsy six days later, the ovaries contained niether corpora hemorrhagica nor corpora lutea.

B. *The intraperitoneal injection of urine.* The realization that the negative character of the results with the hypophyseal transplantation was possibly due to insufficient dosage, prompted a search for a more convenient and more economical source of the anterior lobe substances. The report of Zondek and Ascheim (3) that the urine of pregnant women is capable of

producing biological effects which simulate those of the anterior lobe suggested the use of such urine in these experiments.

Following this suggestion, seven isolated, non-pregnant rabbit does were injected intraperitoneally with 12 cc. of fresh catheter urine from pregnant women, twice daily, for four days. On the sixth day, the injected animals were killed and their ovaries removed for examination. In every ovary large, fresh corpora lutea were seen. The lowest number of corpora lutea in a pair of ovaries following these injections was seven; the highest, thirty-three (fig. 1). Histological examination revealed, however, that these corpora lutea were not the result of ovulation, but of lutein transformation of corpora hemorrhagica with retained ova. The lutein transformation did not reach the same stage in every blood follicle in the same ovary, but varied from that seen in figure 2 to complete luteinization with obliteration of the hemorrhagic center.

In one animal, which had received one single intraperitoneal injection of 12 cc. of urine from a pregnant woman, autopsy six days later revealed only one structure in the pair of ovaries that could be recognized grossly as a corpus luteum. In addition to this, however, there were present in the pair of ovaries fourteen corpora hemorrhagica. Yet, on microscopic examination these corpora hemorrhagica were seen to differ from the ordinary blood follicles occasionally seen in the ovary of a normal rabbit after coitus in that they showed some degree of luteinization (fig. 3).

The twice daily injection of 12 cc. of urine from non-pregnant women for four days was without effect. At autopsy on the sixth day, the nine rabbits so treated presented ovaries with ripe follicles, but without either corpora hemorrhagica or corpora lutea. The injection of two rabbits with specimens of male urine was similarly negative.

Fig. 1. The ovaries from an animal which had received a series of injections of urine from a pregnant woman.

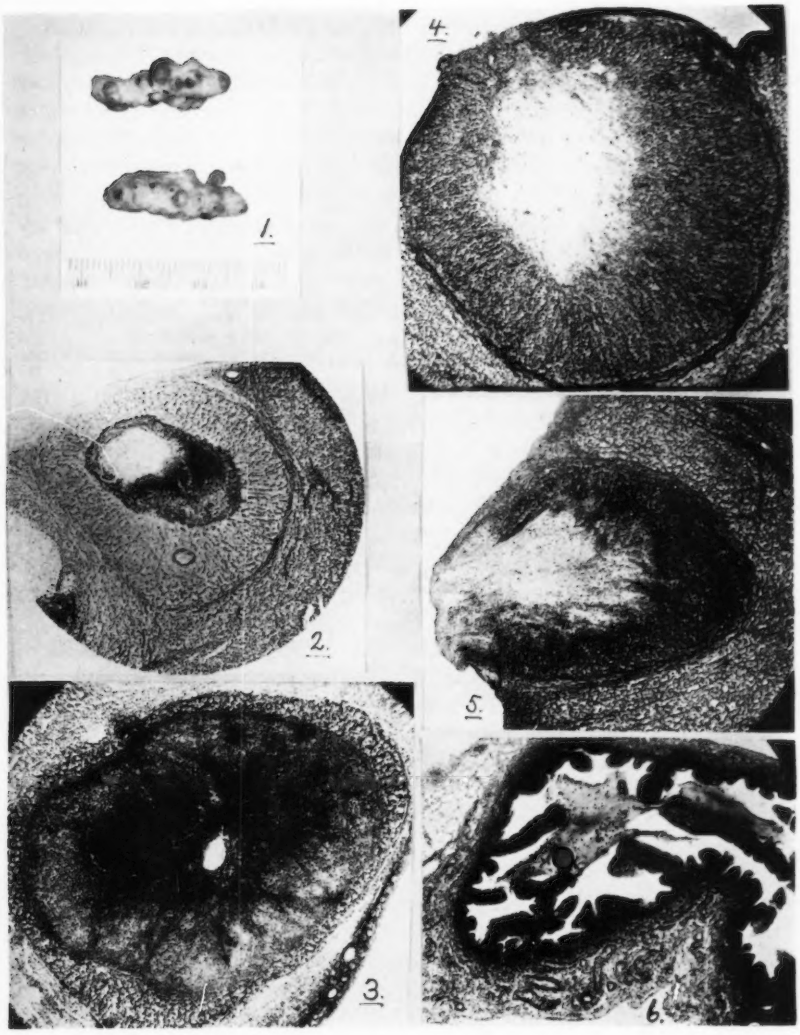
Fig. 2. Microphotograph of a section through the ovary of an animal having received a series of intraperitoneal injections of urine from a pregnant woman, showing a fairly large hemorrhagic center in the corpus luteum. Toward the base of this corpus luteum, the retained ovum may be seen.

Fig. 3. Microphotograph of a partially luteinized blood follicle in an ovary of an animal that had received one intraperitoneal injection of urine from a pregnant woman. In the center of this follicle the degenerating retained ovum may be seen.

Fig. 4. Microphotograph of a corpus luteum in the ovary of a doe autopsied three days after a single intravenous injection of an active urine specimen.

Fig. 5. Similar microphotograph of a fresh corpus luteum in the ovary of a doe autopsied 24 hours after a single intravenous injection of an active urine sample.

Fig. 6. Microphotograph of a section through the fallopian tube of an animal that had been injected with an active urine specimen 24 hours previously, showing the discharged ovum, and the accompanying follicular fluid.



Figs. 1–6

C. *The intravenous injection of urine.* To determine whether or not these effects produced by the intraperitoneal injection of urine could be reproduced by methods which did not involve the possibility of local inflammatory reactions about the ovaries, and to obviate the unavoidable variations in the rate of absorption which intraperitoneal injections entail, a series of isolated, non-pregnant does was subjected to intravenous injections of urine. In these experiments the urine specimens were not obtained by catheterization, the ordinary voided specimens being used without aseptic precautions.

Ten female rabbits, known to be non-pregnant either by exploratory laporatomy or by familiarity with the previous sexual history, were given one single intravenous injection of 5 cc. of urine obtained from pregnant women. One of these does, killed and examined three days afterwards, showed five fresh corpora lutea in the pair of ovaries. From the gross appearance and from the subsequent histological examination (fig. 4), the age of these corpora lutea was calculated to be about two days. In the ovaries of each of the remaining nine rabbits, killed and examined twenty-four hours after the injection, were fresh corpora lutea. The lowest number of corpora found in a pair of ovaries in this series of rabbits was three; the highest, nine. By microscopic examination these corpora lutea were indistinguishable from those resulting from ovulation after coitus, showing distinctly a ruptured wall (fig. 5) and containing no retained ovum.

The fallopian tubes of four of these nine animals were sectioned. In each case, the discharged ova were found (fig. 6).

Two non-pregnant does were injected with specimens obtained from women shortly after labor. One doe, having received 5 cc. of urine obtained 24 hours post-partum, showed no corpora lutea when autopsied 24 hours later. The other doe, injected with a similar amount of a specimen from a woman 21 hours post-partum presented six fresh corpora lutea at autopsy 24 hours later.

As controls, five non-pregnant, isolated does were injected with 5 cc. of urine obtained from non-pregnant women. In no case were corpora lutea found at autopsy on the following day.

Discussion. The results of the experiments with the intravenous injection of urine stand in bold contrast to those in which the urine was injected intraperitoneally. Very recently, Engle (4) has emphasized the differences in ovarian responses elicited by treatment with urine from pregnant women and by freshly implanted anterior lobe. While the implantation of fresh anterior lobe provokes follicular maturation and ovulation in the immature rat ovary, the subcutaneous injection of whole urine from pregnant women results in the luteinization of follicles without ovulation. From these data Engle infers that, "If both the gonad-stimulating factor, and the lutein producing factor are ascribed to the anterior lobe, then it must be assumed

that there are two factors which act on the ovary, or that the same factor acts in a different manner in combination with the urine and as prepared in certain extracts, than when given in the fresh transplant." Our results with the intravenous injection of urine from pregnant women calls to attention another possible factor; that is, the rate of supply of the effective substance, or substances, to the ovary. If this factor is not operative, it would be difficult to explain why the intravenous injection of urine from pregnant women provoked ovulation in the rabbit, while the intraperitoneal injection of similar urine did not, producing instead, the luteinization of the resulting blood follicles.

Regardless of the reason for these differences, however, it is striking that each sample of urine, collected from 18 pregnant women produced, irrespective of the manner of administration, corpora lutea in the injected rabbits, while not one of the sixteen control urines, collected from fourteen non-pregnant women and from two men, had such effect. With two exceptions, the women who furnished the active urine samples were at least eight months pregnant. Yet, these two exceptions are worthy of mention, inasmuch as they were cases of early pregnancy in which certain clinical diagnosis could not be made until some weeks after the urine specimens had been obtained and the injections made. The first of these two cases was a lactating woman whose menstrual periods had come to an abrupt stop three months previously. The other case was one brought to the hospital for pernicious vomiting, having ceased menstruating five weeks previously.

Of the women who furnished the control urines, six were admitted to the maternity ward, suspected of pregnancy at the time the samples were obtained. Since that time, the possibility of pregnancy has been excluded in each case. Of the remaining eight women, one had a mediastinal tumor; another, an ovarian carcinoma. The rest were convalescents in the medical ward. The urines secured from these women were gotten in every stage of the menstrual cycle, including the first day of the menses, when hemorrhage was in progress.

From the small number of cases presented here, one is hardly justified in making any definite statement as to the reliability of this procedure in the rabbit for the diagnosis of pregnancy. Nevertheless, the results so far are decisive. Furthermore, the ease with which these results can be seen by purely gross examination justifies a further study on a statistical basis to determine whether or not this procedure is of sufficient reliability to be of practical clinical use.

SUMMARY

1. It has been impossible to produce ovulation in the rabbit by the transplantation of as many as fifteen fresh rat hypophyses, or by the intraperitoneal injection of twenty-four fresh rat hypophyses.

2. Intraperitoneal injection of urine from pregnant women produces luteinization of the resulting corpora hemorrhagica in the rabbit ovary.

3. A single intravenous injection of urine from pregnant women provokes ovulation in the rabbit.

4. The samples of urine so far obtained from non-pregnant women have been utterly without effect on the rabbit ovary, either when injected intraperitoneally or when injected intravenously.

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Just as this article was ready for press, a personal communication was received from Dr. A. S. Parkes, of London, in which he stated that hypophysectomy (by decerebration, within one hour after coitus prevents ovulation in the rabbit, whereas a similar operation performed later than one hour after coitus does not prevent ovulation. In view of this information it is, perhaps, pertinent that the time elapsing between the intravenous injection of urine from a pregnant woman and ovulation is approximately the same as the normal interval between coitus and ovulation.

THE HETEROGENEOUS TESTIS TRANSPLANT PROBLEM AS APPLIED TO WHITE RATS AND MICE

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Berthold (1849) receives the credit of being the first to obtain definitely positive results with transplanted testis material. The subsequent work in the field has been extensive, but the results conflicting. Moore (1926a) has given a concise review of the literature. The present problem was undertaken to accumulate additional evidence which might help to clarify the status of the heterogeneous transplant problem in man. White rats and mice were selected as experimental animals because they are sufficiently close phylogenetically and can be handled in greater numbers and under conditions much better controlled than is possible with human material.

METHOD. Stock rats of varying ages were used as hosts. They were etherized, shaved over the anterior abdominal wall, painted with mercurochrome, and hemicastrated on the left side, using the peritoneal route (Moore, 1926b). The blood vessels and ducts were ligated high up before cutting. Young adult white mice served as donors. After quickly killing with ether the entire testis was removed. The tunica vaginalis of the rat was everted into the abdominal cavity and the mouse testis was stitched to it by means of a button silk ligature passed through the epididymis pole. The tunica was then replaced in the scrotum thereby pulling the testis graft outside the abdominal cavity. No other treatment was necessary to keep the small graft from being retracted into the abdominal cavity. The incision was closed by a continuous suture through the peritoneum and muscles, and a second one through the skin. The animals after operation received the same treatment as stock rats.

After a period of from five to six months the rats were killed with ether and the grafts were recovered. They were fixed in formalin, cut in 10 micron sections, and mounted serially. The sections were stained with hematoxylin and congo red, and studied histologically for evidences of the efficacy of the graft.

RESULTS. From sixty-three operated rats only forty-three grafts were recovered, since nineteen died during the experiments, and one failed to yield a nodule at the end of the experiment. Of the forty-three grafts

recovered, three remained in the host 185 days; six, 179; nine, 178; five, 165; ten, 164; and ten, 159. Nodules were palpable in these rats throughout the experiment, though there was great individual variation in both the size and firmness in different cases.

Nodules showed great anatomical variations when removed. Some were larger than when transplanted, some smaller. Some were rounded appearing as small testes, others were irregular and were found only because of the silk ligature used to stitch them in place. Figure 1 shows the excellent condition macroscopically of the best graft so far as vascularization is concerned. In other cases, however, there seemed to be no successful vascularization at all.

The microscopical examination of the sections shows that they may be classified into four types:

In type I there is either no definitely positive location of the graft at all, or connective tissue has entirely replaced the old tubules.

In type II occasional areas appear which are made up of the old epithelium. These cells are frequently arranged to give the appearance of tubules separated by large areas of connective tissue.

In type III the graft is slightly or not invaded by connective tissue. Tubules are easily recognized although they show profound degeneration, including a dead epithelium. Sperm heads may be present.

In type IV the epithelium appears to be living though the tubules are atypical, presenting signs of retrogression.

A typical microscopical diagnosis of each type is given in detail:

Type I. Connective tissue, striated muscle and blood vessels are abundant throughout the section. The location of the graft is not indicated though it is possibly suggested by an area of connective tissue on the margin of which many striated muscle fibers end. These fibers are atypical in that they seem to be attached to nothing in particular, but end abruptly. A section of the epididymis is also present. (See fig. 2.)

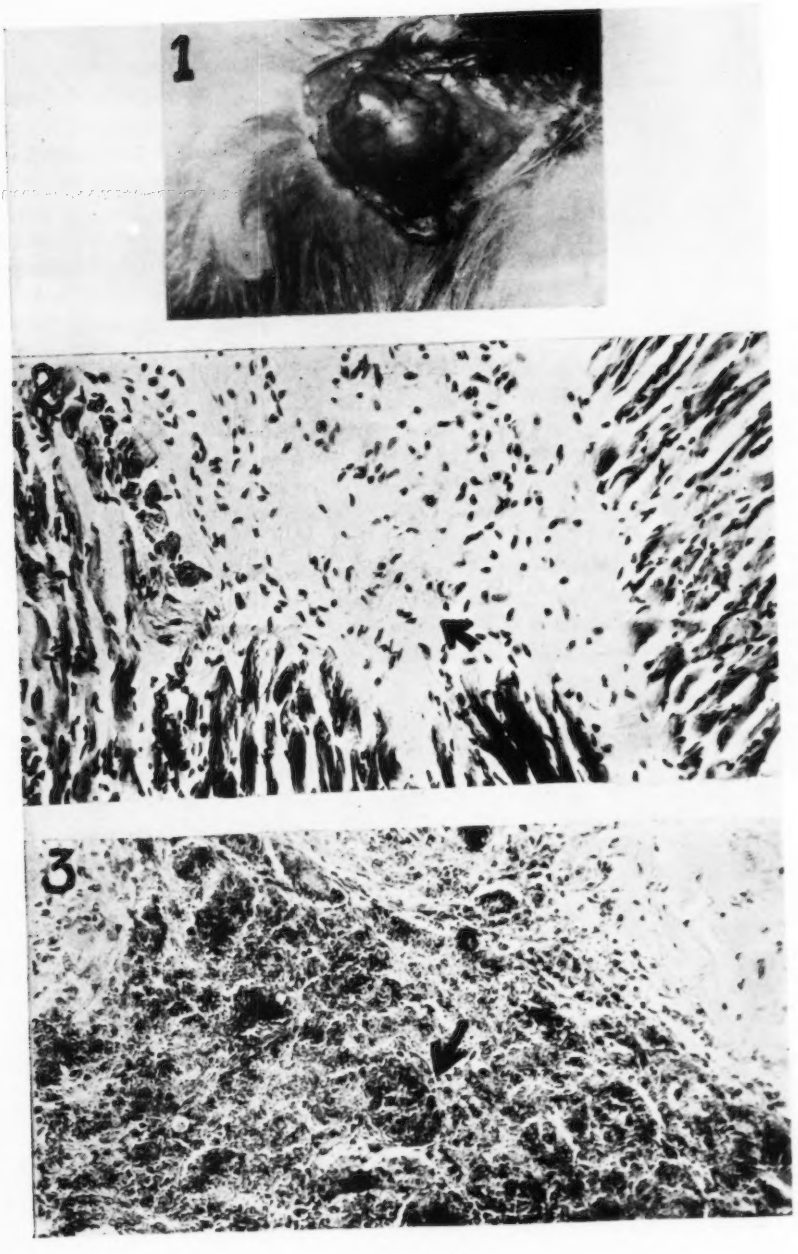
Type II. The area representing the grafted material is surrounded by extra graft muscle, connective tissue and blood vessels. The area itself is being replaced by connective tissue. Large whorls appear to mark the location of some of the old tubules. The cells constituting these whorls contain much brown staining precipitate, evidencing marked degeneration. (See fig. 3.)

Type III. The area of the section representing the graft is egg-shaped.

Fig. 1. The macroscopical appearance of the vascular condition of a graft.

Fig. 2. A type I graft showing no identifiable tubules but with connective tissue whorls possibly marking the old location of the graft. (× 200.)

Fig. 3. A type II graft showing the remains of old tubules whose cells are degenerating. (× 200).



Figs. 1–3

It seems uninvaded by connective tissue though it is well encapsulated by host tissue. The first layer covering it is made up exclusively of fibers. No nuclei or cells are seen. The layer outside of this is equally thick and contains cells with living nuclei. Altogether the capsule appears dense and tight. The middle of the grafted material stains the same color all over. No nuclei can be seen here. The tissue is badly broken and does not show structure. The peripheral row of tubules, however, shows differential staining and is better preserved. In some of these tubules the structure is distinct, though the cells seem to be retrogressing. A region in each of these peripheral tubules, occupied by sperm-heads stains distinctly blue, causing a "zoning" of the tubules by the blue band similar to, but more distinct than the zones in normal tubules. Sometimes this preserved periphery of tubules extends inward to include the second row. Some of the tubules in this second row contain much necrotic material. Going farther down the graft, sperm-heads are found nearer and nearer the center. The connective tissue capsule becomes thinner and thinner, and scar tissue begins to invade the interspaces between the tubules, so that they are more distinctly marked out. In such tubules the epithelium has largely sloughed off. Finally, as the end of the section is approached the tubules showing the sperm heads become less and less evident. (See fig. 4.)

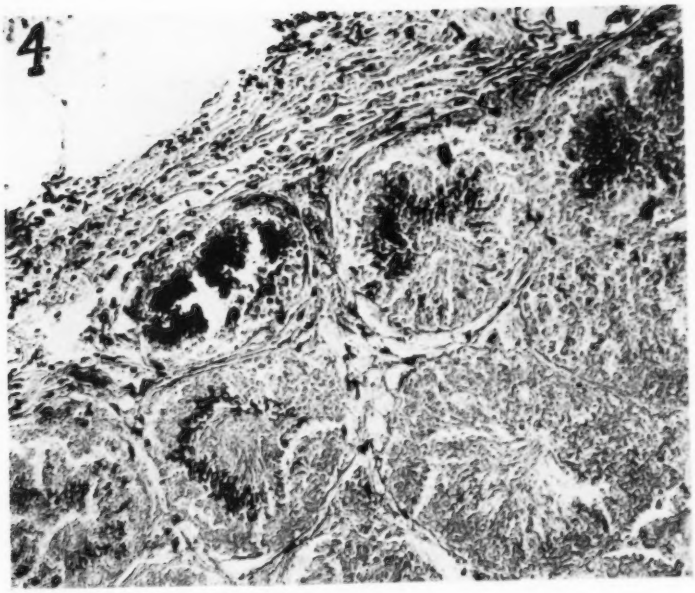
Type IV. Tubules with a well preserved, viable epithelium are present. The lumina, which are abnormally large, contain a pink staining, homogeneous, granular material. There is an entire absence of spermatogenesis. There is little fibrous tissue between the tubules. A section of epididymis is present showing several cross sections because of its tortuous course. It too presents a good epithelium and contains a pink staining granular precipitate, cells, debris, and full formed sperm. In several sections giant cells are found in the epididymis. (See fig. 5.)

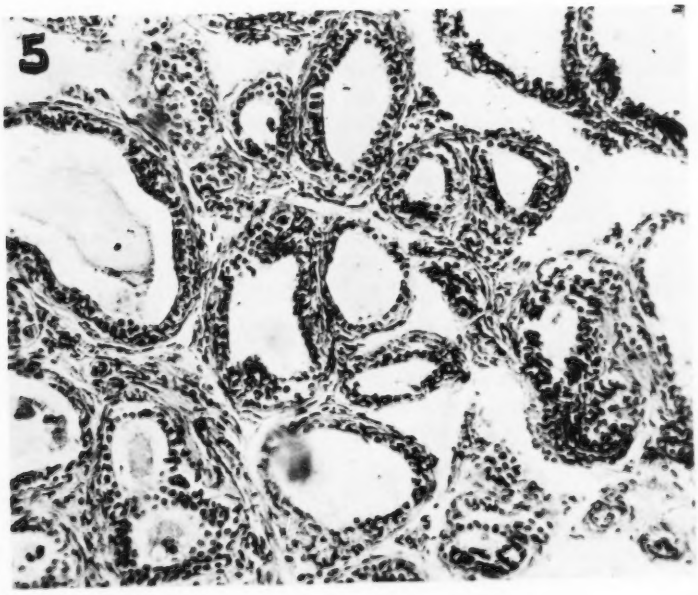
Of the forty-three grafts studied nine or 21 per cent belong to type I, twenty-seven or 63 per cent to type II, six or 14 per cent to type III, and one or 2 per cent to type IV.

Discussion. The strikingly beneficial results reported by Voronoff (1923) from ape testes transplanted into man have aroused an ever ready and credulous sentiment in the minds of many laymen, and a curiosity or alarm, as the case may be, in the minds of scientists. The number of such clinical cases is, of necessity, few, and, even with the patient coöperating

Fig. 4. A type III graft showing the remains of a testis graft in which there are profound retrogressive changes. These are more marked in the middle of the section. The peripheral tubules show remnants of dark staining sperm elements which "zone" these tubules. (× 200.)

Fig. 5. A type IV graft showing viable cells arranged in distinct tubular fashion with very large lumina, empty or filled with a precipitate. Notice the aspermatogenesis. (× 200.)





Figs. 4 and 5

to the utmost, may not be well controlled because of the great rôle which psychic factors play in man. We set out to do the same sort of experiments on animals small enough that large numbers could be used under strictly controlled conditions. We decided to use white rats and mice because phylogenetically they are very much alike, in fact they are much more alike than man and apes because they belong to the same family whereas man and apes belong to different families. Too much emphasis must not be placed on this closeness, however, because serologically rats and mice are not so closely related as man and apes, and from the standpoint of successful grafting of tissues, the serological reactions undoubtedly play a much greater part than do similarities in structure.

The peritoneal route of castration and transplantation is possible in the rat because of the patent inguinal canal. This route insures the least possible disturbance to the blood supply of the scrotal linings, which is important because the absence of interference with this blood supply makes for a quicker vascularization of the graft. The anchoring of the graft outside of the body cavity is necessary for the latter stages of spermatogenesis (Moore and Oslund, 1924).

One hundred and fifty-nine to 185 days was surely sufficient time for the grafts to degenerate or to take. In those cases where nodules persisted it was necessary to select criteria to determine whether the nodule was grafted-donor or scar-host tissue. Most of the differences of opinion of authors in the past arose from the selection of these criteria. One criterion which was used was the sex behavior of the animals. Since sexual activity in the form of attempts at copulation is greater than usual the first few days after castration in the white rat, and since it is a matter of common knowledge that a steer or even a cow will cover another cow in rut, we regarded this type of activity as not exclusively a function of the testis. We, therefore, did not completely castrate the rats and recorded no sexual behavior.

Gross anatomical observations indicate whether or not the graft is represented by the persistence of a nodule made up either of the grafted tissue or of scar tissue. Some knowledge may be gained as to the progress and condition of the vascularity. However, this seems to be of little value in the present experiments because the variation in different cases is marked and because a palpable nodule was present in each of the forty-three cases. Little can be judged from the macroscopical appearance as to the condition of the grafted material. We therefore consider macroscopical criteria unreliable.

By elimination we decided to base our studies exclusively on microscopical findings. The absence of very reliable tests for the measurement of the success of transplantation must be emphasized. Work now in progress by Moore and his co-workers may develop methods which will

make it possible to measure the success more accurately in the future. The four types into which we have classified all the grafts can be divided into two groups: those in which the graft obviously did not take, type I; and those in which the graft may have taken, types II, III and IV. This grouping ignores the possibility that the grafts in group I may have taken and existed for a short time only, being quickly absorbed. It also dismisses the possibility, for which there is some evidence, that these types may simply be successive stages in one process. The situation with reference to types II, III and IV is more elusive than type I. Retterer (1926) in describing a graft taken from one of Voronoff's patients after a year considers the graft positive and functional upon finding a few sections which contain identifiable tubules, although these were not structurally normal. Upon a similar consideration all of our grafts in types II, III and IV would be positive. Stated numerically this would mean that 21 per cent of the grafts did not take and 79 per cent did take. As a matter of fact we do not consider that any of our grafts took. We prefer to regard the grafts in types II, III and IV as having merely persisted. Among the reports of successful testis grafts are those of Moore (1926b) in which the germinal epithelium is viable and spermatogenesis is present. These were homogeneous transplants. In our heterogeneous transplants there were no cases in which the epithelium was normal.

Repeated studies have shown how unreliable are conclusions concerning function drawn from anatomical changes. There is no necessary parallelism between anatomical and functional changes. Experience has shown that usually function suffers from any untoward circumstance more acutely than does structure. This greater fortitude of anatomical changes is shown specifically in the case of the sperm elements. These are clearly not complete sperm in our sections, but only sperm heads. Their presence simply demonstrates their resistence to absorption. Lillie (1919) and Hammond and Asdell (1926) have shown that sperm, both vertebrate and invertebrate, lose their ability to move and to fertilize long before they show any anatomical retrogression. We believe, therefore, that our grafts are in even poorer condition functionally than they appear anatomically. In light of the negative results usually obtained with other heterogeneous transplants and blood transfusions, these negative results are not surprising.

CONCLUSIONS

1. Palpable, well-vascularized nodules showing the persistence of tubules for five to six months may result from mouse testes transplanted into rats.

2. By the criteria of the French workers about 79 per cent of these grafts are viable and functional, but by the criteria we have adopted they have simply persisted anatomically and are degenerating.

The author wishes to express his gratitude to Dr. A. J. Carlson for his unstinted interest in the supervision of this work, and to Dr. Carl R. Moore for expert diagnosis of the microscopical preparations.

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THE EFFECTS OF DEGENERATIVE SECTION OF THE VAGI AND THE SPLANCHNICS ON THE DIGESTIVE TRACT

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Although many workers have observed the behavior of the bowel during stimulation of the vagi and splanchnics or immediately after their section, almost nothing has been done in the way of studying the properties of the gut; that is, its irritability, rhythmicity, latent period and ability to conduct stimuli and rush waves, after degenerative section of the extrinsic nerves. It was only as this paper was being written that Nolf's monograph appeared with its interesting conclusions in regard to the structure of the enteric nervous system of the chicken. He appears to be the only one so far to have entered into the field that we have been exploring. Years ago it was touched upon slightly by Langley and Magnus when they cut some of the mesenteric nerves and later studied a few of the reactions of the bowel.

Especially now that surgeons are learning to relieve spastic paralysis of the extremities, Raynaud's disease, Hirschsprung's disease, arthritis deformans, angina pectoris, and hyperacidity by section of one or more of the autonomic nerves, it is highly desirable that physiologists and clinicians should know more about what happens in the abdomen when these nerves are cut and allowed to degenerate.

EXPERIMENTAL RESULTS. *Effects of stimulation of the vagi on different parts of the bowel.* Before we proceeded to a study of the denervated bowel we performed a few experiments to see what effect stimulation of the vagi would have on different parts of the small intestine. Rabbits alone were used. They were anesthetized with urethane, the lower dorsal and lumbar cord was pithed and the abdomen was opened under salt solution. The movements of the bowel were recorded in a way which has been described elsewhere (Alvarez and Mahoney). While these records were being obtained, either the right or the left vagus was stimulated in the neck with the weakest faradic tetanizing current that would produce an effect on the gut.

So far as we could see, there was no pronounced difference between the effects obtained with the two vagi and no peculiarity in the type of response

obtained in different parts of the bowel. The effect of stimulation was that described by other observers; that is, either a transient increase in the amplitude of the rhythmic contractions, or else transient inhibition of one or two contractions, or a slight drop in tone, and then a transient rise in tone and an increase in the amplitude of the rhythmic contractions.

In the few experiments done, no effect was obtained with the constant galvanic current even when it was strong enough to injure the nerve, but a marked rise in the amplitude of the contractions was obtained when the stimulus was interrupted several times a second.

Types of experiment in which nerves were cut. Our experiments may be divided into five groups: 1, those in which after vagotomy three weeks or more were allowed to pass before the animal was opened and studied; 2, those in which from five to twenty days elapsed; 3, those in which the vagi were cut just before the animal was opened in the tank of warm saline solution; 4, those in which both major splanchnic nerves were cut, and 5, those in which both vagi and both major splanchnics were cut.

VAGOTOMY WITH DEGENERATION OF PART OF AUERBACH'S PLEXUS. *Mortality.* With the rabbits under ether anesthesia the two vagi were cut just below the diaphragm. The completeness of the section was checked later at necropsy. During the study we operated on seventy-two animals. Seven more were kindly prepared for us by Doctor Mann and his assistants. Of the seventy-two, thirty-one (43 per cent) died. Four others would almost certainly have died if we had tried to keep them three weeks, so the mortality was more nearly 49 per cent.

In an effort to improve matters we kept the incoming animals for a few weeks, feeding them well and weeding out the weaklings and those heavily infested with parasites. Sometimes we used only local anesthesia and we worked rapidly so as to complete the operation in fifteen minutes. We withheld food for two days after operation and then fed the animals ourselves, but in spite of all our care the mortality continued unchanged.

That this mortality was not due simply to the opening of the abdomen was shown by the fact that many of the animals continued to go down hill long after they should have recovered from the immediate effects of this operation. One animal which was allowed to live seventy-four days never regained its weight; another which lived sixty-six days remained sickly and lost 41 per cent of its original weight, and another which lived sixty-five days died of diarrhea and paralysis of the hindquarters.

In many of the animals no obvious cause for death could be discovered at necropsy. Pneumonia was found in seven, paralysis of the hindquarters in one, and perforating gastric ulcer in two. Peritonitis was never seen. In seven, death seemed to be due to diarrhea and inanition. As will be seen later, these conditions resulted from a great increase in the irritability of the digestive tract. With the removal of inhibition, the large cecum, so

important in the slow digestion of cellulose, was emptied, and the animals were probably handicapped much as they are when this organ is closed off surgically. As will be shown later, peristalsis was so active that food was apparently rushed out of the bowel before digestion and absorption could take place.

No one could watch these animals without being impressed with the fact that in the rabbit vagotomy is a serious operation and one which, for a time at least, so disturbs nutrition that only the strongest animals can survive. Possibly we would have saved more than we did if we had known of Nolf's experience with chickens. He found that after vagotomy the gizzard loses its power of trituration, so that the animals have to be fed soft mushes. It is suggestive that the muscular part of the rabbit's stomach is also flabby after vagotomy, and it may be that more animals could have been tided over if rough foods had been withheld from them. Mann tells us that his mortality was similar to ours until he made the rabbits fast for a few days before operation; then more recovered.

That some rabbits can promptly adapt themselves to the handicap was shown by the fact that seven of the thirty-one which lived for more than three weeks regained the weight that was lost immediately after operation. One, which lived a year, became fat and healthy and another which lived three months gained in weight in spite of the fact that the stomach contained ulcers.

A few also of the experimenters who have performed subdiaphragmatic vagotomy in dogs have had difficulties in keeping the animals alive for the first two weeks after operation; others, however, speak of the operation as if it were innocuous. Actually, we would expect it to be less harmful in a carnivorous than in an herbivorous animal because in the former, digestion takes place more quickly. In man the cutting or destruction of one vagus does not seem to have much if any deleterious effect and bilateral vagotomy has been done many times either purposefully or incidentally during high resections of the stomach.

Thirty-one animals lived more than three weeks after the operation and were then studied with the abdomen open in a tank of warm salt solution. Twenty-one were allowed to live for from three to four weeks; six for a month, one for sixty-six days, one for seventy-four days, one for three months, and one for a year.

The minimal period of three weeks was chosen because it was thought that by that time degeneration of the nerve fibers would be complete. S. E. Johnson, who has made a particular study of this subject, kindly examined segments of stomach and gut from some of our animals, and reported the same marked degeneration of the fibers in Auerbach's plexus that he had observed previously in dogs with the vagi cut. Under such circumstances we expected to find some disturbances in conduction of

waves and stimuli along the bowel. We did not expect to find much if any disturbance in the local rhythmic contractions because practically all the available evidence indicates now that the muscle can, if necessary, contract of itself or with the help of neurones situated in the wall of the bowel (Alvarez, Gasser, van Esveld, Nolf).

Activity of the bowel after vagotomy. Contrary to expectations derived from reading the reports of other investigators, we found that when the animals were anesthetized with urethane, the middle region of the cord destroyed, and the abdomen opened under warm salt solution, the bowel was almost always more active than normal. Not only were the local segmenting movements very active but rushes were frequent and easily started.

It is common to find "normal" animals which show little activity when opened in the tank, but few of the animals with vagi cut acted that way. The three that showed a quiet or normally active bowel were all sickly. Animals prepared as we prepare them for study in the tank usually live from nine in the morning until late in the afternoon, or until the end of the experiment when they are killed. Sickly, non-resistant animals die early, sometimes within an hour. During this time the bowel sometimes remains quiet. That it is only inhibited and not injured in any way is indicated by the fact that when it is excised from the body and placed in warm oxygenated Ringer's solution it often contracts normally. We were interested therefore to see that even in the animals with the vagi cut and the splanchnics blocked by destruction of the cord, poor health brought about an inhibition which disappeared when the bowel was excised. This inhibition was probably brought about by the post-ganglionic sympathetic fibers which were not affected by section of the splanchnics.

Most of the animals that survived long enough to be studied in the tank had not had diarrhea or had had it and recovered, and most of them showed hard fecal balls in the colon. Aside from the tendency to emptiness, the cecum did not show any constant abnormality.

The stomach. In many of the rabbits with both vagi cut the pars pylorica of the stomach was flabby and almost empty but in others it appeared to be normal. The fact that the stomach was apparently normal in some of the animals that had recovered from the operation and the fact that some starved rabbits presented an empty, flabby pars pylorica made us suspect that the lack of tone might sometimes be due at least in part to starvation. It is suggestive, however, that in the chicken the gizzard which corresponds to the pars pylorica in mammals is greatly weakened by vagotomy (Nolf).

Our impression is that gastric peristalsis was more active and the waves deeper in the vagotomized animals than in normal controls. Certain it is that in a few the waves were unusually deep. We made a few records

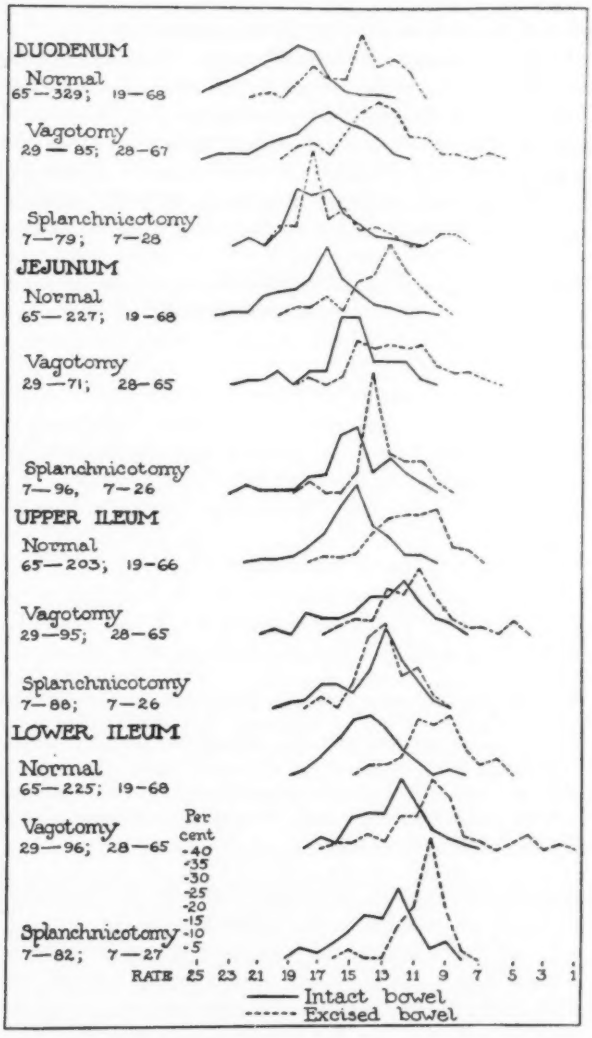

Fig. 1. Percentage distribution polygons showing rates of rhythmic contraction in intact and excised bowel from normal animals and animals with vagi and splanchnics cut. In each set of two curves the ordinates represent percentages and the abscissas the number of contractions in each minute. The figures connected by dashes represent first the number of animals used and second the number of records studied.

of gastric peristalsis in the animals with the vagi cut and found the same peculiarities that have been observed in normal rabbits.

Rate of rhythmic contraction in the small bowel. The local rhythmic movements of the small bowel were generally more active in vagotomized than in normal animals and the amplitude was large.

The polygons in figure 1 represent percentage distributions of the rates in four different parts of the intact and excised bowel, in normal animals, in animals with the vagi cut, and in animals with the splanchnics cut. It will be noted that in every case the rates are graded from high values in

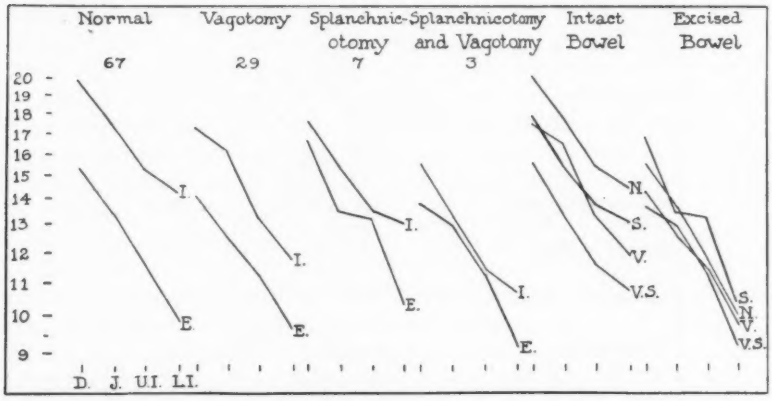

Fig. 2. Mean rates of rhythmic contraction in four different parts of the intact and excised bowel showing the effects of vagotomy and splanchnicotomy. In each of the six sets of curves the four abscissas represent duodenum, jejunum, upper ileum, and lower ileum. The figures *67*, *29*, *7*, and *3* represent the number of animals used. The letters *I* and *E* represent intact and excised bowel. *N* represents normal bowel, *S* bowel after splanchnicotomy, *V* bowel after vagotomy, and *V S* bowel after vagotomy and splanchnicotomy.

the duodenum to low ones in the ileum. This is shown again in figure 2 where the means are plotted on semilogarithmic paper.

In figure 2 it will be seen that excision caused a decrease in the rate which was of the same degree (23 per cent) in duodenum, jejunum, and upper ileum. In the lower ileum, however, the decrease was 31 per cent of the mean for the intact bowel. In the fourth column it will be noted that a similar decrease in rate, again most marked in the lower ileum, was brought about by degenerative section of the vagi. These observations, together with some made by Nolf (pp. 462 and 473), may perhaps be explained in the following way. In embryonic life, Auerbach's plexus grows from above downward. Perhaps because of this fact, the number of fibers and

ganglion cells diminishes progressively from the duodenum to the lower ileum. It may be, then, that any injury, such as is wrought by the trauma of excision from the body, or vagotomy, will leave the muscle in the distal end of the bowel with a very poor nerve supply. In the cat, Klee found that stimulation of the vagi had most effect on the duodenum and least on the lower ileum. In some animals it had no effect on the ileum.

As will be noted in figure 1, and in the fifth column in figure 2, almost all the slowing in the rate of rhythmic contraction that is observed after vagotomy takes place in the intact bowel. With excised segments there is no shifting of the modes of the distributions, and the only reason for a slight downward shift of the means is that in a few animals some very slow rates were observed, rates slower than any ever seen in normal controls. In two instances segments of the lower ileum would not contract at all.

It should be noted, however, that this slowing was not always definite, and in twenty of the twenty-nine vagotomized animals in which the rates of rhythmic contraction were counted they were within the limits of normal. In the one rabbit that lived for a year the rates in the four different parts of the intact bowel were those represented by the modes of the "normal" curves in figure 1.

In most of the vagotomized animals there was little change in the gradient in rate of rhythmic contraction and in only three was it obliterated. In one animal the rate in the ileum was actually higher than normal.

It may be worth noting that in one cat, shortly after the vagi were cut (below the diaphragm), the rate in the jejunum fell to ten a minute and the gradient from duodenum to lower ileum could be expressed by the figures 17, 10, 15, and 12. Later the rate in the jejunum rose to its usual level between fifteen and eighteen a minute. This observation is suggestive because anatomists have found that a large part of one vagus runs directly to a point in the upper jejunum.

To sum up then, the vagus appears to exert some influence in maintaining the normal rate of rhythmic contraction in the intact bowel. In most of the experiments vagotomy had no influence on the rate of contraction in the excised bowel.

Peristaltic rush. In the normal rabbit opened in the tank, rushes appear either spontaneously during the day or else whenever water is introduced into the esophagus or whenever the animal is made to swallow. In the vagotomized animals rushes appeared more frequently than normal and they started either spontaneously or after the slightest artificial stimulus. In twenty of the twenty-three animals in which our records are explicit on this point, there were many rushes, and in twelve there were more than we have ever seen in normal rabbits. As already pointed out, this appears

to be the explanation for the fact that so many of the animals that failed to recover were emaciated and suffering with diarrhea.

Our impression was that although more rushes started in the duodenum, fewer than normal went through to the ileocecal sphincter. Some tended to slow up and stop after they had gone half way down the bowel. Figure 3 represents in percentage distribution polygons the rates of travel of over a hundred rushes in sixteen normal and seven vagotomized animals It will be noted in the vagotomized rabbits that while a number of the waves that started in the duodenum with a normal rate of travel slowed down in the

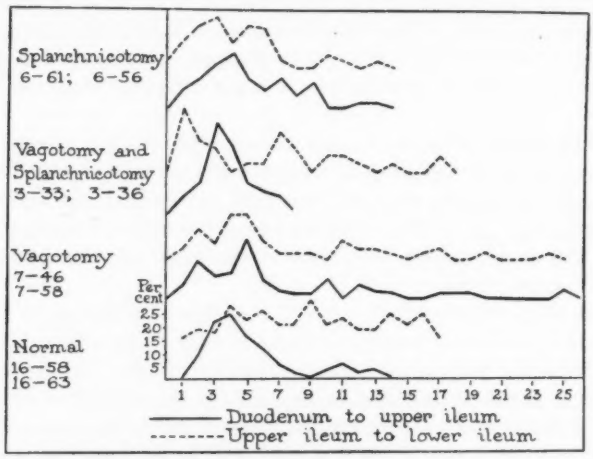

Fig. 3. Percentage distribution polygons showing the rate of travel of peristaltic rushes from duodenum to upper ileum and from upper ileum to lower ileum. In each set of polygons the ordinates represent the number of observations in percentages of the total and the abscissas the rates of travel in centimeters a second. The figures connected by dashes represent the number of animals and the number of records studied.

lower part of the ileum, others went on and accelerated just as they normally do.

When one obtains simultaneous records of the activities of six or more parts of the intestine of an animal opened under salt solution one finds here and there evidence that rises or drops in tone or amplitude of contraction took place almost simultaneously in duodenum and lower ileum. Obviously some stimulus must have traveled rapidly either through Auerbach's plexus or, what seems to us more probable, along the extrinsic nerves which are to be found in the mesentery. We rather expected to find this type of rapid conduction impaired or lost after degenerative

section of the extrinsic nerves of the bowel, so we were surprised to find it so well preserved. It appeared to be present not only after the vagi had been cut, but also after the splanchnics had been cut and even when both sets of nerves had been cut together. The changes in the records can hardly be artifacts due to movements of the animal or of the intestine as a whole because such movements produce twitches which are synchronous in all the records and usually are easily recognizable.

Evidently, then, some highly efficient conducting paths remain even after degenerative section of the extrinsic nerves of the bowel and destruction of most of the spinal cord.

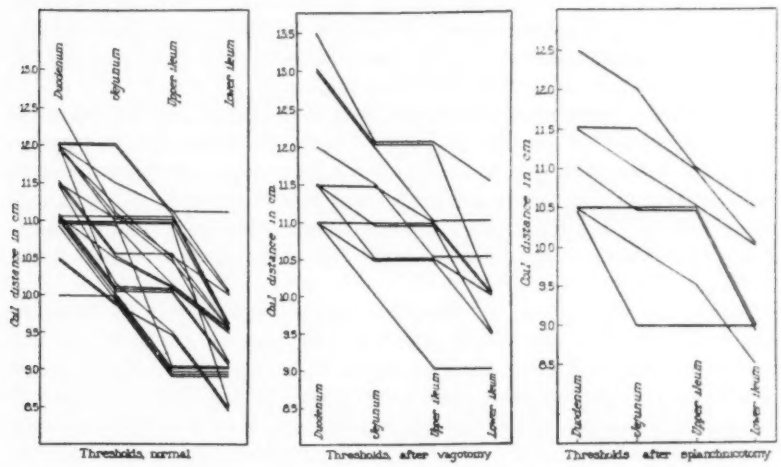

Fig. 4. The gradient of irritability in normal rabbits and in rabbits with the vagi and splanchnics cut.

Conduction of stimuli along the bowel. If the bowel is stimulated at any point with a faradic tetanizing current, wavelets spread out on either side. Normally they go farther caudad than orad and they probably go a little faster caudad. Measurements are hard to make on account of the ever-present tendency of the bowel to spontaneous rhythmic contraction. Furthermore, it is not always easy to average the results because there may perhaps be different types of conduction along the bowel with different rates. As a result, the distribution polygons of the measurements are generally wide and flat and without any definite mode.

In the normal intact bowel, measurable records showing wavelets traveling out 5 cm. or more from the point stimulated were obtained twenty-seven times in every 100 attempts. In the excised bowel such conduction

was observed forty-seven times in each 100 attempts. As will be seen from the tables published in a recent paper (Alvarez and Hosoi, 1929), it was as easy after vagotomy to demonstrate conduction in the intact animal as in excised segments, perhaps because some inhibition had been removed.

Normally more waves were measured traveling caudad than orad, and as will be seen from the tables mentioned in the preceding paragraph this relation was maintained after vagotomy. The big difference appeared in the lower ileum where the ratio of wavelets traveling orad to wavelets traveling caudad was 1:3.3. In other parts of the bowel it was about 1:1.8. After vagotomy conduction was poor in the lower ileum, both in the intact animal and the excised segments. This fits with other observations already described, and all point to a particular vulnerability of the lower part of the bowel to degenerative section of the vagi.

The rates of conduction in different parts of the intact and excised bowel were unchanged by vagotomy. Considering the difficulties and uncertainty of measurements, it is remarkable how close the several means were. (The actual figures are given in a recent paper by Alvarez and Hosoi, 1929.) The gradient in rate of conduction in the several segments from duodenum to lower ileum was also left unchanged by section of the nerves.

The failure of vagotomy to affect the rate of conduction in the bowel of the rabbit is what one would expect if Nolf's views are correct. His studies on the bowel of the chicken suggest that conduction along the bowel is effected by neurones which are not involved in degenerative section of the extrinsic nerves. Somewhat against this view is the fact, to be brought out later, that degenerative section of the splanchnics greatly shortened the distance over which the conduction of stimuli can be demonstrated.

Irritability of the bowel. Figure 4 shows that after vagotomy, the irritability of the bowel as measured by faradic tetanic stimuli, was sometimes normal and at other times increased. The technic used in these measurements has been described in a recent article.

Latent period. Figures 5 and 6 show that vagotomy had a tendency to increase slightly the number of slow responses with faradic stimuli in the upper part of the bowel, and especially in the intact jejunum. As will be seen from figure 7 the mean latent periods in the four regions in the intact bowel with faradic stimuli are almost the same in normal and vagotomized animals. In the excised bowel they were the same except in the duodenum where the mean for the vagotomized animals was 0.23 second as compared with 0.20 second in the normal animal.

The lines in figure 7 show also that the mean latent period in all parts of the bowel was shorter with galvanic than with faradic stimuli. It is curious also that galvanic stimuli brought out, in normal and vagotomized

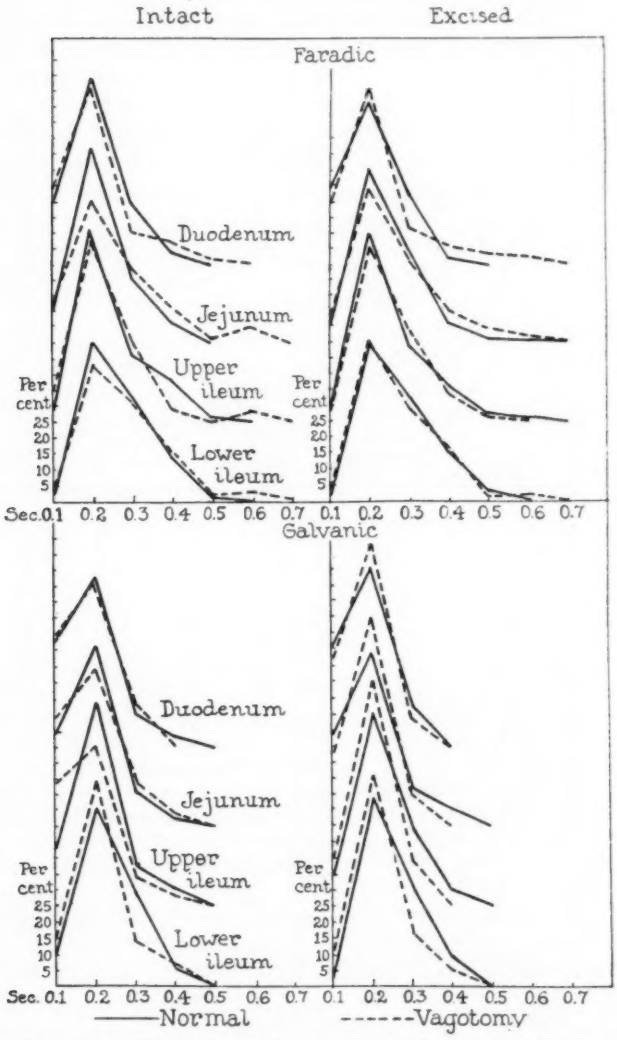

Fig. 5. Percentage distribution polygons showing latent periods obtained with faradic stimulation in the intact and excised bowel of normal and vagotomized animals. In each set of polygons the ordinates represent the number of observations in percentages of the total. The abscissas represent the latent periods in tenths of a second.

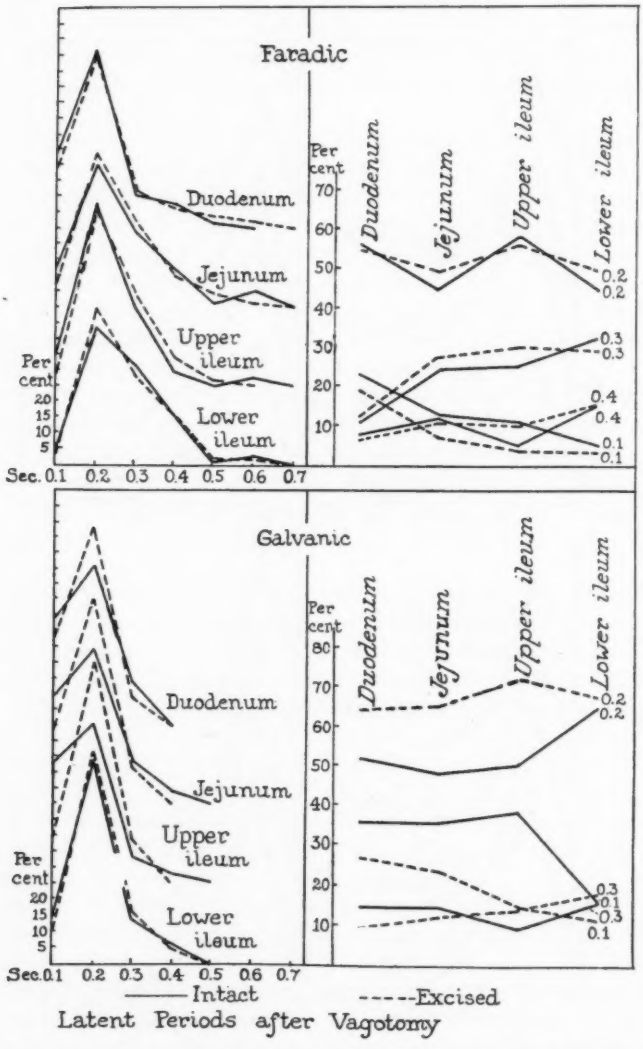

Fig. 6. Percentage distribution polygons showing latent period in vagotomized animals The lines on the right show for four parts of the bowel the incidence in percentages of the different lengths of latent period observed. The figures are the same as those plotted in a different way on the left.

animals, definite differences in the behavior of the upper and lower parts of the bowel. After vagotomy the mean galvanic latent period was shortened in the ileum while with faradic stimuli it was somewhat lengthened in duodenum and jejunum.

The upper half of figure 6 shows that with faradic stimuli there was in the vagotomized animals almost no difference in the behavior of the intact and excised bowel. With galvanic stimuli there was a big difference in the upper three-fourths of the bowel where excision of the gut brought

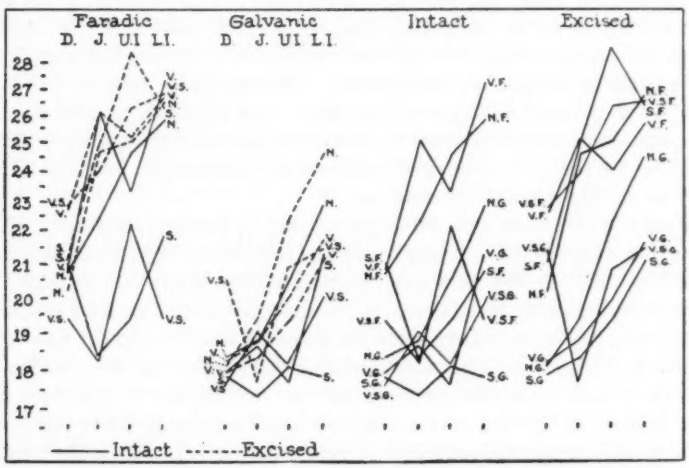

Fig. 7. The lines plotted on semi-logarithmic paper represent mean latent periods in four different parts of the intact and excised bowel, with faradic and galvanic stimuli, in normal animals, and in animals with the vagi and splanchnics cut. In each of the four sets of lines the four abscissas represent duodenum, jejunum, upper ileum, and lower ileum. The ordinates represent hundredths of seconds. *N* represents normal, *V* vagotomy, *S* splanchnicotomy, and *V S* vagotomy and splanchnicotomy. *F* represents faradic and *G* galvanic.

about a great reduction in the number of 0.1 second reactions. This was so well compensated by an increase in the number of 0.2 second reactions that the means for duodenum and jejunum were unchanged. The distribution polygons for intact and excised ileum with both faradic and galvanic stimuli were almost exactly the same. The lower part of figure 6 shows again that in vagotomized animals the latent periods with galvanic stimuli were shorter than those with faradic stimuli and that the difference was most marked in the lower part of the bowel.

So far we can offer little explanation for these differences. The shorter latent period with galvanic current may be due to the greater efficiency of

this type of stimulus when applied to a sluggish type of muscle. The shorter periods in the lower part of the bowel after vagotomy might be due to the removal of nervous inhibition. Other differences between the behavior of different parts of the bowel with faradic and galvanic stimuli may be due to a preponderant or unique effect of the latter on the muscle and a preponderant effect of the former on the enteric nerves. What information we have gotten so far indicates that stimulation of the vagi or of the nerves in the mesentery with the uninterrupted galvanic current has little if any effect on the bowel. Furthermore, with faradic stimulation applied directly to the wall of the gut, the curve of contraction is made up of two components while with galvanic stimulation there is but one.

The effects of atropin and epinephrine. Because of the relation known to exist between vagal endings and atropine, and sympathetic endings and epinephrine, we were interested to note that excised segments of the intestine in which most of Auerbach's plexus had degenerated responded normally to minute doses of these drugs.

OBSERVATIONS MADE LESS THAN THREE WEEKS AFTER VAGOTOMY. Nine animals were studied at intervals varying from three to sixteen days after operation. The flabby pars pylorica and overly active gut so typical of vagotomized animals were observed as early as four days after section of the nerves. In some experiments the stomach was full and the wall tonic, but this was often true in animals that survived longer than three weeks. In some there was not much rhythmic activity in the gut. In six there were many rushes but the rate of travel did not accelerate in the lower bowel as it so often does in normal animals. Usually the rates of rhythmic contraction in the several regions of the bowel were normal, but in three it was notable that the duodenum was contracting slowly, and in one it was contracting more rapidly than normal. Excised segments contracted normally except in one case in which the rates were all unusually slow. With rare exceptions the rates in intact and excised gut were within the limits of normal.

Our impression, then, is that there is a removal of inhibition from the bowel, a slowing of the rate of rhythmic contraction, and a loss of tone in the stomach within a few days after vagotomy, but these changes are less marked and less constant than they are after degeneration of the nerve endings has become complete.

Vagotomy performed immediately before the animal was opened in the tank. As several rabbits under urethane anesthesia died when the vagi were cut we found it advisable first to block the nerves with a little procain. Strange to say, no such difficulty was encountered when the animals were operated on under ether. Some animals were lost at first because the salt solution in the tank invaded the mediastinal and pleural cavities by way

of the sheath of the esophagus, but soon we learned to avoid this by keeping the thorax of the animal above the level of the fluid.

After overcoming these difficulties, we studied thirteen animals. In nine, vagotomy seemed to have no effect. In three of them the bowel was observed before and after cutting the nerves and no difference could be seen in its behavior. In one instance while the writing levers attached to the bowel were tracing their way over the smoked drum the nerves were cut and we were surprised at the absence of any change in the record; there was nothing to indicate that anything had happened. In three animals the rhythmic movements were unusually active, suggesting that vagotomy had had some effect in removing inhibition. In another rabbit in which both the vagi and the splanchnics were cut, the bowel was very active. In one there was much stagnation of material in the lower ileum, suggesting the presence of spasm in the ileocecal sphincter.

The rate of rhythmic contraction in the intact bowel was unchanged in the duodenum but somewhat slowed in the jejunum and ileum. In the excised bowel the rates were normal except in the ileum where they averaged a little higher than usual.

These results are so nearly within the range of normal that little can be said about them. Certainly no increase in activity was seen comparable to that generally observed in the rabbits with degenerative section of the nerves. The lesson that was impressed on us was that it would be unwise to accept unreservedly, as many do, conclusions in regard to the function of the vagi based on observations made immediately after their section. Such results should always be compared with those obtained after degeneration of the fibers.

DEGENERATIVE SECTION OF BOTH SPLANCHNIC NERVES. *Mortality.* After such skill had been attained that both major splanchnics could be cut in a few minutes, we operated on seventeen rabbits. Young thin animals were used because the absence of fat makes it so much easier to find the nerves. Of the seventeen, ten died and one other had to be studied on the ninth day when its hind legs became paralyzed. The mortality was therefore 65 per cent. In two, the cause of death was peritonitis but in the others necropsy showed nothing but emaciation. In one of those that died and in five of those that lived gastric ulcers were present. They must have formed rapidly because a number were present in the stomach of an animal that died two days after the operation.

Some of the animals refused to eat for a few days before they died, and in every one the bowel was abnormally empty as if food had been rushed out too fast. Vagotomy was hard enough on the rabbits, but splanchnicotomy was even worse. Strange to say, the loss of weight was not so marked after splanchnicotomy as after vagotomy, and all the animals that lived soon regained what they had lost and went on with their growth. All those that

lived looked healthy, ate well, and had hard feces. Diarrhea was noted in only one of the animals that died.

Appearance of stomach and bowel. The animals that lived were studied after nine, twenty-two, twenty-three, thirty, thirty-one, thirty-three and fifty-three days. On opening the abdomen we sometimes observed marked reddening of the cecum and slight reddening of the small bowel due to dilatation of blood vessels. This reddening was present in the animal that lived fifty-three days.

The stomach was full and firm in some of the animals and a little softer than normal in others. Just as in normal rabbits gastric peristalsis was sometimes present and sometimes absent. The rhythmic movements in the small bowel were always active; perhaps as active as in vagotomized animals, but comparisons are hard to make. Judging from the statements in textbooks of physiology we had expected to find the movements more active after splanchnicotomy than after vagotomy. The great irritability of denervated Vella loops in dogs has been commented on by Benczur.

Rushes appeared about as frequently as they do in normal animals and not as frequently as in vagotomized animals. This was somewhat surprising because after splanchnicotomy the bowel was unusually sensitive even to a light touch. When it was picked up between the fingers it often contracted down so as to form a hard white cord. In most of the animals the cecum was normally filled and in all of them the colon contained formed feces.

Rate of rhythmic contraction in the small bowel. The polygons in figure 1 show one of the most surprising observations made during this study; that is, that there was little if any difference between the rates of rhythmic contraction in the intact and excised bowel. In figure 2 it will be seen that the approximation of the lines representing mean rates is due to the fact that these rates, particularly in the upper part of the bowel were slow in the intact animal and faster than normal in the excised segments. The gradient in rate of rhythmic contraction remained unchanged.

In the fourth column of figure 2 it will be noted that in the intact bowel the depressant effect of splanchnicotomy on the rate of rhythmic contraction was the same from duodenum to ileum. The effect of vagotomy was the same as that of splanchnicotomy except at the lower end of the ileum where with vagotomy the depression was more marked. In the fifth column it will be seen again that in the excised bowel splanchnicotomy increased the rate of contraction over what it was in normal segments. Why this should be we do not know.

Peristaltic rush. While watching the rushes it seemed to us that some were fast and some unusually slow. Figure 3, which represents actual measurements, shows that while in the upper bowel the rate of travel of these waves was normal, in the lower bowel it was often abnormally slow.

For this reason the upper distribution polygon which represents rates of travel in the lower half of the bowel is narrower than normal and centered more to the left.

Conduction of stimuli along the bowel. It will be remembered that in normal animals stimulation with faradic current at any point of the gut generally produced wavelets that ran orad and caudad. In normal animals 100 stimuli produced twenty-seven records of waves spreading out at least 5 cm. in one or both directions. In animals with the splanchnics cut 100 stimuli produced only eleven such records which shows that conduction was impaired. In the excised bowel where normally 100 stimuli produced forty-seven good records of conduction, splanchnicotomy reduced the number to ten, and in addition made it impossible for us ever to get signs of conduction 10 cm. orad or caudad from the point stimulated. In the intact bowel the poorest conduction after splanchnicotomy was observed in the upper and lower ileum; in the excised bowel the only signs of conduction were seen at the two ends of the bowel.

In the intact bowel the normal preponderance of caudad conduction was observed, and the ratio of ascending to descending waves was 1:7. Little, however, can be said about these differences because our observations were too few. Actually in the seven animals only thirty-one records of conduction were obtained. Under the circumstances it was useless to calculate mean rates of conduction because the figures would have had little value.

To sum up, then, conduction, which was much improved by vagotomy, was made much worse by splanchnicotomy.

Irritability of the bowel. It will be seen from figure 4 that the normal gradation in the thresholds for faradic stimulation remained after splanchnicotomy. The means for the four different parts of the bowel were practically the same as those obtained in normal animals. This surprised us because when we noticed how sensitive the bowel was to the touch we expected it to be highly sensitive also to the faradic current.

The latent period. It will be seen from figure 7 that degenerative section of the spanchnics had a profound effect on the mean latent period in all parts of the bowel except the duodenum. The lines representing the data from splanchnicotomized animals stand out by themselves, away from the others which represent measurements on normal and vagotomized animals. With one exception, the actual figures were shorter than normal, which is what one would expect with such an irritable bowel. The exception was in the excised bowel where, with faradic stimuli, the latent periods were either normal or somewhat lengthened. With galvanic stimuli there was a good gradient in the excised segments but, strange to say, there was none in the intact bowel. In both intact and excised bowel the gradation obtained with faradic stimuli was irregular.

In figure 8 it will be seen again that the largest differences between the

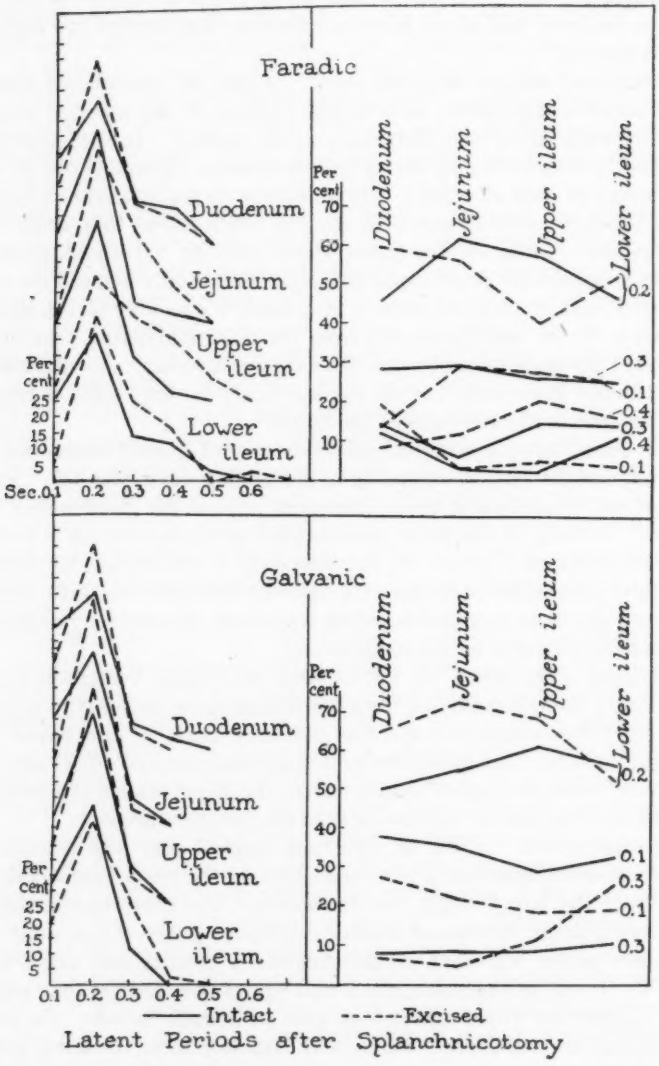

Fig. 8. Percentage distribution polygons showing latent periods in different parts of the bowel after splanchnicotomy. The lines on the right show for four parts of the bowel, the incidence in percentages of the different lengths of latent period observed. The figures are the same as those plotted in a different way on the left.

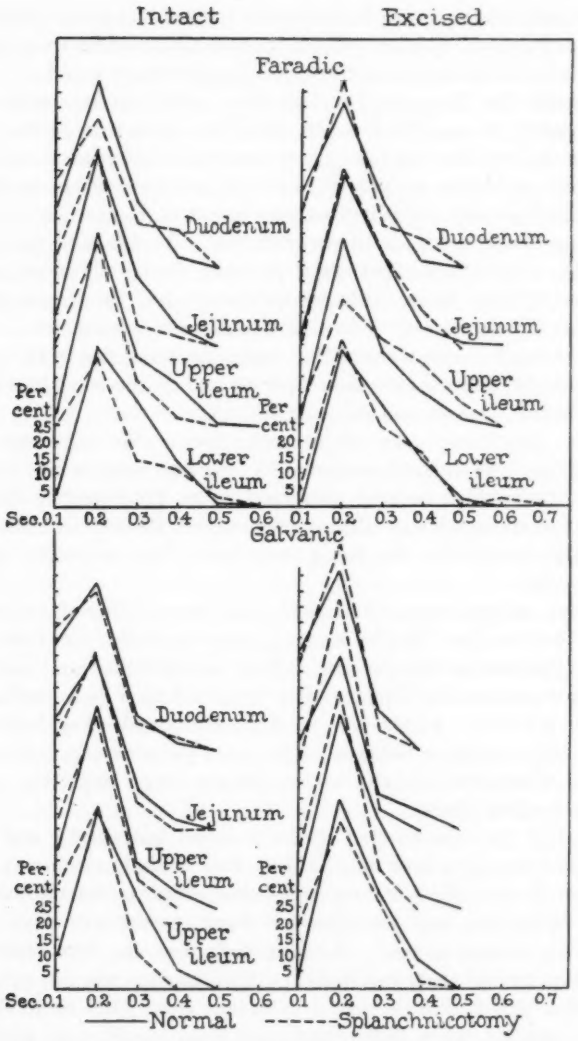

Fig. 9. Percentage distribution polygons showing differences in latent period in intact and excised bowel and with faradic and galvanic stimuli after splanchnicotomy.

results with intact and excised bowel were obtained with faradic stimuli. Figure 9 shows that the greatest difference between the results with fara-

dic and galvanic stimuli were obtained with excised bowel and particularly with the upper ileum. It shows also in more detail the differences between the reactions of the normal and the splanchnicotomized bowel.

It is probable that if we could explain these variations from normal after splanchnicotomy we could tell much about the structure of the enteric nervous system and the physiologic relations between muscle and nerve. Unfortunately, we know so little that it does not seem to us worth while even to hazard guesses. Such theorizing would be dangerous even if we could be sure of the accuracy of every datum. Although we have at our disposal 751 observations, they were obtained from only seven animals and they had to be so divided into groups that each of the sixteen distribution polygons represents only from thirty to sixty measurements.

All that we can feel sure about is that degenerative section of the splanchnics has a marked effect on the latent period and on the ways in which the bowel responds to faradic and galvanic stimuli.

COMBINED VAGOTOMY AND SPLANCHNICOTOMY. We performed combined vagotomy and splanchnicotomy on nineteen rabbits and lost fourteen. One other which became paralyzed in its hindquarters had to be studied on the sixteenth day after operation and another was studied on the ninth day because it was going down hill. The mortality therefore was 84 per cent.

Most of the animals succumbed in the first two or three days, and only four of the fourteen that died before they could be studied lived for about a fortnight. Pneumonia was present in four and diarrhea and emaciation seemed to account for death in six. One scratched open its wound and died on the eleventh day. In two the stomach contained several ulcers and in another the mucous membrane of the pars pylorica was hemorrhagic. In another, which was allowed to live twenty-seven days, the stomach contained a healing ulcer.

In several of the animals that died the bowel was empty and so contracted as to resemble a hard white cord. One animal, which was allowed to live a month, was still suffering with severe diarrhea but the two others that lived thirty-one and twenty-seven days respectively were healthy and had fairly normal stools. The fecal balls were dry but smaller than normal. The animal that was studied after nine days was not eating well. It looked sick and it had decreased in weight from 1420 to 1050 grams. One of the animals, when killed, had more than regained its weight and the others had returned almost to normal. The animal that lived sixteen days was well until its hindquarters became paralyzed. We regret now that we did not make histologic studies of the spinal cords in these animals to see why so many become paralyzed after vagotomy and splanchnicotomy.

On opening the abdomen the bowel in every case was found to be ex-

tremely active and in fact more so than it ever is in normal animals or perhaps even in animals with degenerative section of the vagi or the splanchnics. Not only were the rhythmic movements active but peristaltic rushes were frequent. The stomach was active in three out of the five animals that were studied. In one it was larger than normal, in two it was full and hard to the touch, and in two the pars pylorica was empty and flabby as it often was after vagotomy alone. This was probably due to the fact that these two animals were the ones that were sick and not eating well. In several of the stomachs we noted peculiar spasmodic contractions in the pars pylorica or in the segment just above it.

The colon in two of the animals was full of gas, and in all of them it was unusually active and almost empty. The cecum was always empty. Usually it was reddish in color due to dilatation of the blood vessels.

Rhythmic contractions. In all of the animals the rates of rhythmic contraction in the intact bowel were much shower than normal. They were slow after vagotomy, slower after splanchnicotomy, and slowest after section of both sets of nerves. The modes of the distribution polygons showing the number of waves a minute were, from duodenum to lower ileum, 15.5, 13.0, 12.0 and 11.5. The gradient was good in all the animals. It was good also in excised segments, and the rates were practically normal. On account of the great slowing in the rate in the intact bowel there was, as in the splanchnicotomized animals, practically no difference between the rates in the intact and the excised gut.

Peristaltic rush. In the two healthiest animals the rushes traveled at a practically normal rate from one end of the bowel to the other. In the sicklier rabbits the waves traveled more slowly.

Conduction of stimuli. Conduction was poor and in this respect the behavior was more like that after splanchnicotomy than after vagotomy. So few records of conduction could be obtained that little can be said about the rates except that they were within limits of normal. In the intact animals there were three measurable orad waves to seventeen caudad and in the excised segments there were two orad to six caudad.

Irritability. Thresholds for faradic stimuli were studied in three animals. They were normal in duodenum, jejunum, and upper ileum and slightly lower than normal (coil distance 10.5 cm. instead of 9.5 cm.) in the lower ileum.

Latent period. This was measured in only three animals. Figure 7 shows that the latent periods tended to be short and more like those in splanchnicotomized than like those in vagotomized animals. Moreover, the gradients in intact and excised gut were irregular and flattened much as they were after splanchnicotomy. We are not publishng the distribution polygons because they are based on so few cases that we cannot be sure about the significance of the many peculiarities that were observed.

Peristaltic rush at the time of death. When a rabbit dies and the tissues become cyanotic, a peristaltic rush generally sweeps down the bowel. This might be due to an increase in the carbon dioxide content of the blood, acting locally on the muscle, or it might be due to stimuli coming down the nerves from the dying brain and cord. The fact that we saw agonal rushes in a number of the animals with both vagi and splanchnics cut makes it probable that changes in the bowel itself are responsible for the phenomenon.

COMMENT. As one would expect after section of the vagi the animals seldom sighed or moved the forepaws as they normally do when rushes travel down the bowel. Occasionally after vagotomy we saw signs which suggested that messages from the bowel were traveling to the brain or upper cord by way of the sympathetic chain, but we could not be sure.

The marked slowing of the rhythmic contractions after degeneration of the extrinsic nerves is of interest in connection with the fact that a similar slowing appears immediately after the death of the animal (Ascanio and Alvarez, 1929). For years one of us (Alvarez) has looked upon this agonal slowing as a result of poor oxidation and a lowered rate of metabolism but now we have to consider the possibility that it may be due to the deprivation of some tonic influence from the vagi and splanchnics. Against this explanation is the fact that a marked decrease in rate did not appear immediately after section of the vagi. In one animal with both vagi and both splanchnics cut just before the abdomen was opened in the bath of salt solution, the rate of rhythmic contraction remained normal. Immediately after death, however, the rates in jejunum and upper ileum were almost halved while that in the duodenum remained unchanged and that in the lower ileum became faster. The bowel in toto was then removed from the animal and left in the tank of salt solution, which was aerated. The rates in all parts of the bowel were again markedly slowed. Segments were then cut out and transferred to a beaker of warm aerated Locke's solution where the rates became faster, perhaps because of the better oxidation. They were, however, still slower than those observed in the intact animal after degenerative section of the nerves. It appears, therefore, that the rate of oxidation is a factor in determining the rate of rhythmic contraction. This subject will be discussed more in detail in a paper soon to be published by Ascanio and Alvarez.

The observations described in this paper do not agree with some of those reported in the past. It may be that the functions of the vagi and splanchnics are different in rabbits from what they are in dogs and cats, but it is probable that some at least of the discrepancies are due to the fact that no one has hitherto obtained graphic records such as we now analyze, and few have waited for degeneration of the nerve-endings. As we have shown, this produces a picture very different from that seen immediately after section of the nerves.

In the rabbit, both the vagi and the splanchnics certainly serve as inhibitors or brakes to keep the bowel from reacting too powerfully to every stimulus. So far as we could see there was little sign of any antagonistic relation or balance between the two sets of nerves. When both were cut the bowel became even more irritable and reactive than it was when either alone was destroyed. The only place where we observed a big difference was in the faculty of conduction. We have always suspected that the shadowy but imposing structure of vagotonic and sympathicotonic theory reared by Eppinger and Hess was based on flimsy and inadequate foundations but we never knew until now how flimsy they were.

In view of the fact that there are a number of functional disturbances of the stomach and bowel which conceivably might be relieved by vagotomy or splanchnicotomy, we hope later to direct more effort toward learning the causes for the high mortality observed by us. It may well be that methods can be found for tiding the animals over the period during which adjustment must be made for the loss of the regulatory nerves.

SUMMARY

The irritability, rhythmicity, and latent period of the gut, and its ability to conduct stimuli and rush waves, have been studied after degenerative section of the vagi, the splanchnics, and both vagi and splanchnics together. Rabbits were used.

Stimulation of one vagus in the neck with a weak faradic current produced either excitation or transient inhibition followed by excitation. These effects were about the same in all parts of the bowel. A constant galvanic current did not have any effect but a marked response was obtained when the current was interrupted by a rapid opening and closing of the circuit key.

Vagotomy resulted in death of 49 per cent of the rabbits operated on. Many recovered from the immediate effects of the operation, but nevertheless continued to lose weight and go down hill. In several death was due to diarrhea and inanition, produced by the hypermotility of the bowel and the emptying of the cecum. Some recovered and lived for months in good health. Gastric ulcers were found in six.

Thirty-one animals lived for more than three weeks. The abdomen was opened under a bath of warm salt solution, the lower spinal cord was pithed and records were obtained of the activity of several parts of the bowel. Peristalsis and rhythmic contractions were always unusually active.

In many of the animals the pars pylorica of the stomach was flabby and almost empty; in others it appeared to be normal. Much of this difference probably depended on the amount of food that the animal had been eating. Gastric peristalsis was a little more active than normal and the waves were sometimes unusually deep.

In the small bowel, and particularly in the lower ileum, the rates of rhythmic contraction were slowed by vagotomy. In two instances segments of lower ileum did not contract rhythmically at all. In only three animals was the gradient in rate of rhythmic contraction from duodenum to ileum flattened or reversed. In the excised bowel vagotomy had little influence on the rates of contraction.

The rate of travel of peristaltic rushes was practically unchanged by vagotomy. In the normal animal excision of the bowel makes it much easier to demonstrate conduction of stimuli; in vagotomized animals there was no difference after excision, probably because inhibition had already been removed by the operation, and conduction in the intact bowel was as good as it usually is in normal excised segments. Normally it is easier to demonstrate conduction caudad than orad and this was true after vagotomy.

After vagotomy the distance over which conduction of stimuli could be demonstrated was shortened in the lower ileum, both intact and excised. This and other observations suggest that the lower ileum, with a somewhat attenuated myenteric plexus, suffers most from vagotomy.

The rates of conduction of stimuli in different parts of the intact and excised bowel were unchanged by vagotomy. The vagotomized bowel was abnormally sensitive to faradic stimuli but the normal gradient of irritability from duodenum to lower ileum remained unchanged. Vagotomy made a number of changes in the latent periods observed in different parts of the bowel after faradic and galvanic stimuli. It did not alter the reaction of the bowel to atropin and epinephrin.

Nine rabbits were studied at intervals varying from three to sixteen days after vagotomy. A flabby pars pylorica was observed as early as four days after operation. The removal of inhibition from the bowel was less marked and less constant than it was when sufficient time was allowed for degeneration of the nerve-fibers.

Thirteen rabbits were studied immediately after vagotomy. In nine there appeared to be no effect from the operation and in the others there was little definite change from normal.

In seventeen rabbits both major splanchnics were cut and allowed to degenerate. The mortality was 65 per cent. In the animals that died the bowel was abnormally empty, and in many death appeared to be due to inanition. Gastric ulcers were found in six.

The cecum was the only place in which dilatation of the blood vessels was observed. The stomach was generally normal. The intestine was overly active, but there were fewer rush waves than there were in vagotomized animals. The bowel was so sensitive that when touched it often contracted so as to form a firm white cord.

The rates of rhythmic contraction were very slow in the intact bowel

and slightly faster than normal in excised segments. The gradient in rate of rhythmic contraction from duodenum to ileum remained unchanged.

Peristaltic rushes traveled at a normal rate in most of the splanchnicotomized rabbits. Conduction of stimuli was so difficult to show in both intact and excised bowel that little can be said about the rates.

The thresholds for faradic stimuli in the different parts of the bowel were practically normal.

Splanchnicotomy shortened the latent periods in all parts of the bowel except the duodenum. In the intact bowel with galvanic stimuli, the normal gradient in latent period from duodenum to ileum was obliterated. There were many differences between the effects of faradic and galvanic stimuli.

In nineteen animals both vagi and both major splanchnics were cut and allowed to degenerate. The mortality was 84 per cent. Diarrhea and emaciation accounted for death in half of the animals. Gastric ulcers were found in three of the nineteen, and in another the mucous membrane of the stomach was hemorrhagic. A number of the animals in which the vagi or splanchnics or both were cut became paralyzed in the hindquarters.

In the animals which lived three weeks or more the bowel was found to to be extremely active. The cecum was always empty. The rates of rhythmic contraction were markedly slowed. Peristaltic rushes traveled normally except in sickly animals. Conduction of stimuli was poor. The thresholds for faradic current were practically normal. The latent periods were markedly changed and shortened much as they were after splanchnicotomy alone.

It appears, then, that at least in the rabbit, both the vagi and the splanchnics serve as inhibitors and regulators of intestinal activity. The only sign of an antagonism between the sympathetics and the parasympathetics was found during the study of conduction, which was improved by vagotomy and injured by splanchnicotomy. The gastro-intestinal tube possesses a large degree of autonomy and the neuro-muscular mechanisms responsible for orderly diastalsis must be looked for in the bowel itself.

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STUDIES ON THE SO-CALLED HEART HORMONE

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From the time of Haller in the eighteenth century, the question of the nature of the stimulus initiating the heart beat has aroused the interest of physiologists. The discovery in 1848 of the existence of nerve ganglia in the heart itself led for a time to the idea that these nerve centres are responsible for the rhythmic activity of the heart. Later, however, the work of Gaskell, Engelmann, and others indicated that cardiac muscle can contract rhythmically apart from nerve structures. Some inner stimulus, then, must be postulated as acting rhythmically in the heart muscle itself, or perhaps as acting continuously, but producing a rhythmic response of the muscle fibre. Of recent years, since the importance of chemical factors in the regulation of tissue activity has been shown, it has been suggested that the initiation of the heart beat may be dependent upon a hormone-like substance.

In 1922, Demoor (1922a) showed that extracts of auricular tissue of the dog's heart have a stimulating effect on the isolated perfused heart of the rabbit. On the isolated auricle, also, he (1924) found that an extract of the sino-auricular node or of the right auricle produced an augmenting effect, while an extract of the left auricle caused a diminished beat, and a ventricular extract had a toxic action. This led to the suggestion that the heart beat is regulated by a "humoral" substance produced chiefly in the sino-auricular node and right auricle. This view has been developed by L. Haberlandt (1924), who obtained, with the frog heart, similar augmenting and accelerating effects with extracts of sinus and right auricle. The active principle he called "sinus-hormone," but later, since he found it not only in the sinus but also in the auricle, auriculo-ventricular ring, and base of the ventricle, he (1925) suggested the name "automatic substance" or "heart-hormone." This he believes to be the normal stimulus for the spontaneous heart beat, the constant excitation producing a rhythmic effect owing to the refractory period of cardiac muscle (Haberlandt, 1926a). He suggests that the hormone should be of great use clinically as soon as it can be produced in sufficient quantity and purity.

It is noteworthy, however, that Haberlandt obtained positive effects in about 50 per cent only of his experiments with heart extracts. More-

over, he gives no control experiments with extracts of tissues other than the heart, to support his contention that this is a specific hormone.

The present research was undertaken, therefore, to investigate the specificity of this stimulating and activating substance, and to throw light upon its possible value as a therapeutic cardiac stimulant.

METHOD. With the exception of nine experiments on the surviving frog heart, this investigation has been made entirely on the isolated heart of the rabbit, perfused from the aorta with oxygenated Locke-Ringer solution at 38°C. Fifty-two such experiments were performed. The apparatus used was a modification of the Langendorff apparatus, in which the temperature of the perfusing fluid is completely unaffected by the rate of flow. The beats of one auricle and one ventricle were recorded on a kymograph in the usual way. Injection of extracts was made by means of a hypodermic needle through the rubber tubing close to the perfusion cannula.

The extracts of the tissues to be investigated were made by a method similar to that adopted by Demoor (1922b). The tissues were obtained from the animal as soon as possible after death, and a weighed amount was ground thoroughly to pulp with a small quantity of Locke's fluid and clean sea-sand. After trituration, the paste was diluted further in the proportion of 1 gram of tissue to 15 cc. (or in later experiments, 10 cc.) of Locke's solution. The mixture was allowed to extract for several hours (generally overnight, for about 18 hours) in a refrigerator, was then centrifuged, and two or more cubic centimeters of the clear fluid used for injection.

In some cases an alternative extraction was made in a similar way with 95 per cent alcohol. The alcoholic mixture was extracted for 18 hours in a corked flask, was centrifugalized, and the supernatant fluid allowed to evaporate to dryness at room temperature. The residue was then extracted with a quantity of Locke's solution equal in amount to the original alcoholic extract.

The extracts were warmed exactly to the temperature of the perfusing solution before injection, and were injected slowly so as to cause no rise of pressure in the aorta or coronary system.

The tissues investigated were the sino-auricular node, the right and left auricles, right and left auricular appendages, right ventricular wall, and the base and apex of the left ventricle. Control experiments were also performed on extracts made from tissues other than the heart: including striped muscle, from thigh, diaphragm, and upper end of esophagus: smooth muscle from intestine, stomach, and lower end of esophagus, liver, lung, and brain.

The tissues were obtained from rabbit, cat, dog, sheep, and calf, and all the extracts were tested on the rabbit heart. Extracts of frog tissues were tested on the frog heart.

RESULTS. *Rabbit extracts.* The results with extracts of rabbit heart were variable, as was also noted by Demoor and Rijlandt (1925). Thirteen experiments were performed. In six of these slight augmentation was observed on injection of extracts of sino-auricular node; the effects were small but definite. In only two cases was there a very slight acceleration. Similar slight augmentation was obtained with extracts of right and left auricles in about 40 per cent of the cases, and with extracts of left ventricle in about 33 per cent. In other experiments the extracts either were inactive or caused diminution of the heart beat. Toxic effects, i.e., diminution and slowing of beat, were also frequently produced as an after effect following an augmentation. Alcoholic extracts produced similar but less marked effects. These results were compared with those obtained on injection of extracts of other tissues. A slight augmentation was found with two out of three injections of extract of skeletal muscle, with a single injection of liver extract, and with one out of two injections of brain extract.

Cat extracts. In five experiments, in which extracts of cat tissue were tested on the rabbit heart, a temporary augmentation was produced in every case by extracts of sino-auricular node, right auricle, and left ventricle. These augmentor effects were more marked than with rabbit extracts, but were followed almost invariably by a very marked toxic action, causing profound depression of the beat of both auricle and ventricle, sometimes even a standstill lasting for many minutes. Injection of extracts of skeletal (thigh) muscle were made in three experiments, and caused slight augmentation but no after-depression.

Dog extracts. Five experiments were performed on the rabbit heart with extracts of dog's heart. The effects were slight and variable. Extracts of the sino-auricular region gave slight slowly developing augmentation in three of the cases, and similar results were obtained with extracts of the right auricle, except that the augmentation was even less marked. Slight toxic after-effects were sometimes observed, but these were much less than with cat extracts.

Sheep extracts. The results in seven experiments with extracts of sheep's tissue were much more definite. Extracts of sino-auricular node, right and left auricles, and right and left ventricles showed augmentor and sometimes accelerator action, often quite marked, but there was little difference in the effect of extracts from different parts of the heart. Ventricle, whether right or left, base or apex, seemed just as active as auricle or the sinus region. The augmentor action was greater and the toxic after-effects were much less than with extracts from rabbit, cat, or dog.

Fig. 1. Effect of extract of right auricle (atrium) of calf on the beat of rabbit heart.

Fig. 2. Effect of extract of right auricular appendix of calf on the beat of rabbit heart.

Fig. 3. Effect of extract of left ventricle of calf on the beat of rabbit heart.

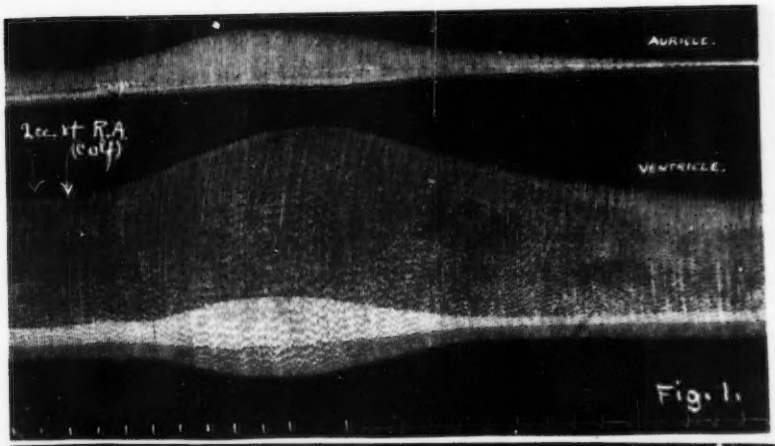


Fig. 1.

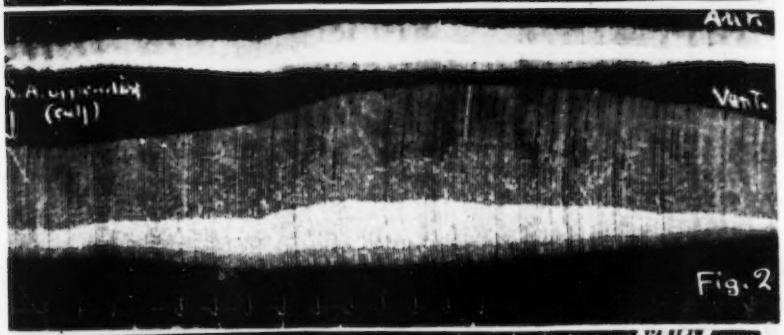


Fig. 2.

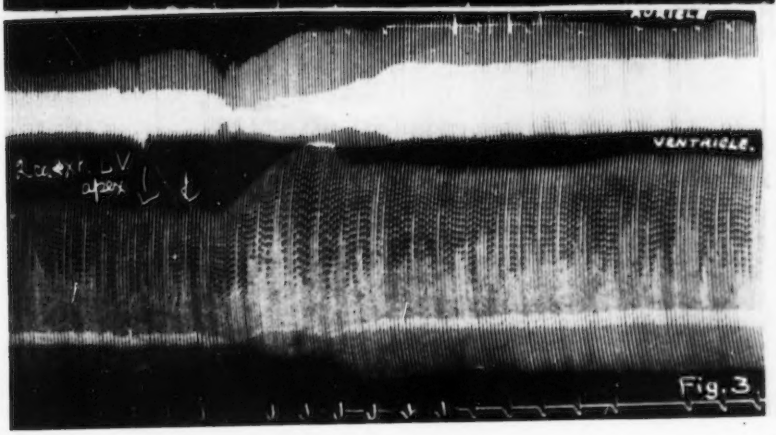
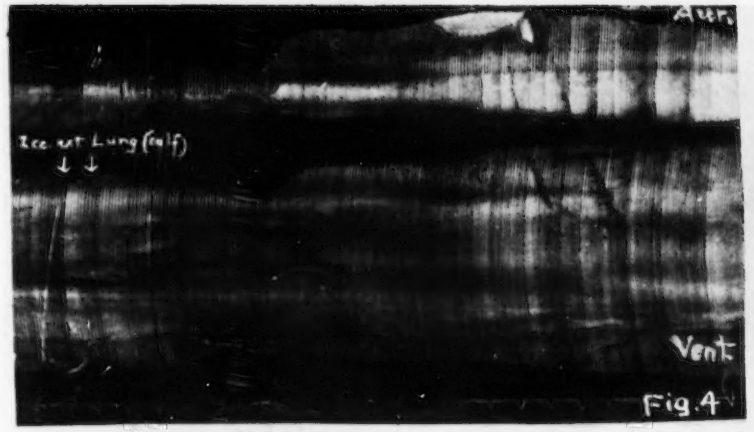
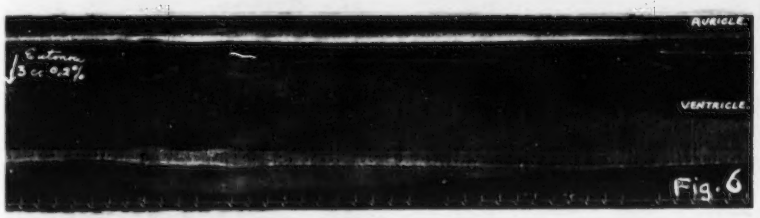


Fig. 3.





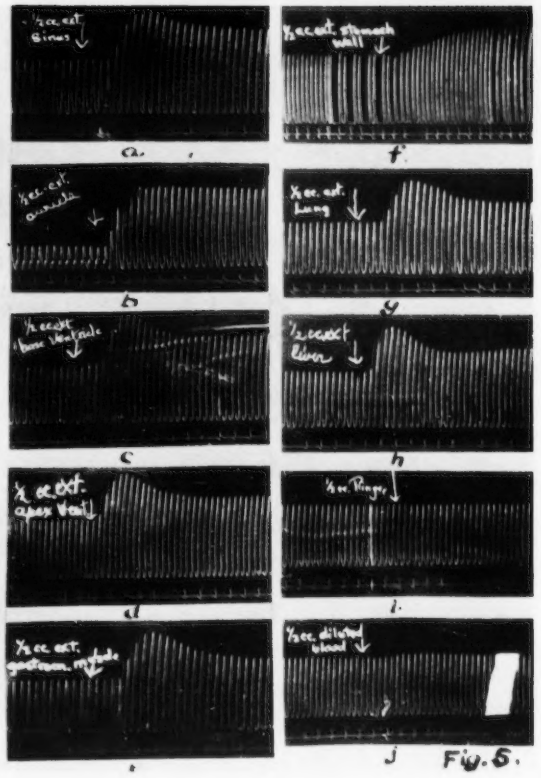


Fig. 4. Effect of extract of calf lung on the beat of rabbit heart.

Fig. 5. Effect of extracts of frog's tissues on the beat of frog ventricle. a, extract of sinus venosus; b, extract of auricle; c, extract of base of ventricle; d, extract of apex of ventricle; e, extract of skeletal muscle; f. extract of smooth muscle; g, extract of lung; h, extract of liver; i, Ringer solution; j, blood diluted with Ringer solution.

Fig. 6. Effect of "eutonon" (0.006 gram).

Three injections were also made of extracts of skeletal muscle (diaphragm), which in two cases produced an augmentation of the heart beat, but less marked than with the heart extracts.

Alcoholic extracts also showed similar effects, but much smaller than with the saline extracts.

Calf extracts. Twenty-two experiments were performed with extracts from the heart and other tissues of the calf, the effects being much more impressive and clear-cut than with extracts from other animals. Extracts of any part of the heart produced an almost invariable augmentation and slight acceleration (figs. 1–3). There was no indication that the sino-auricular region was more active than other parts, in fact it usually appeared to be slightly less active than the right auricle, and in one or two experiments where the region of the node was isolated with extra care, so as to include the least possible amount of auricular muscle, the augmentor action seemed to be less than in other cases. The auricular appendages were as active as the atria (figs. 1 and 2) and the activity of the left auricle was as great as that of the right. Both right and left ventricles contained an active substance, the effect being, perhaps, a little less marked than that of the auricles (fig. 3). All ventricular tissue, however, seemed active, whether taken from right or left ventricle, base, or apex, and whether an endocardial surface was included or not.

Extracts of calf tissue other than heart were also tested, and found to produce augmentation, though somewhat less marked. In 11 out of 16 experiments, extracts of striped muscle produced an augmentation, occasionally, but not invariably, followed by a slight diminution of beat. The striped muscle was in most cases of the red variety, obtained from the diaphragm, since in procuring calf heart from the slaughter house, diaphragmatic muscle always remained attached, and formed a convenient source of the tissue. In a few experiments pale muscle from the thigh was tested, and in one experiment striped muscle from the upper part of the esophagus. No difference in action could be shown between extracts of these different varieties. Smooth muscle was obtained from the lower end of the esophagus, and from the intestine, care being taken to include only muscle, and none of the mucosa. In one case smooth muscle from the wall of the superior vena cava was tested. In all these experiments a slight but definite augmentation was shown. Extract of liver also gave an augmentor effect, while lung extract had a particularly constant effect, producing both an increased amplitude and an accelerated rate (fig. 4).

The effect of heat on the active substance was also investigated, since Haberlandt and Demoor are in disagreement on this question of thermostability. Demoor and Rijlandt (1925) state that the active substance of the sino-auricular node is destroyed by heat above 70°C., while Haberlandt (1926b) finds that his frog "hormone" is thermostabile. In the present

Ringer solution; j, blood diluted with Ringer solution.
Fig. 6. Effect of "eutonon" (0.006 gram).

research it seemed that heat has little or no effect upon the active substance, since the action of the extracts was apparently unimpaired by heating, even to boiling, for five minutes.

The activity of the extracts varies, however, according to the length of time they are kept in solution after extraction. On keeping for two or three days an increased augmentor action is usually shown, decreasing after the third day, and disappearing completely in from 6 to 10 days. If toxic effects have been shown at first, these are reduced by keeping the solution for two or three days. These variations may perhaps be due to the existence of two active substances in the extracts, one of which has a toxic effect on the heart beat, and the other an augmentor action. The toxic substance may perhaps decompose first, thus causing either a lessened toxic action or an increased augmentor effect. Later, the augmentor substance also disappears, and the extract becomes inactive.

In a few experiments the effect was investigated of continued perfusion with the extract diluted ten times further, but the results were variable and inconclusive. No convincing augmentation could be shown in these cases.

Frog extracts on frog heart. Nine experiments were done on the frog heart, perfused with a 0.6 per cent Ringer's solution through a Straub cannula. Extracts were made of sinus venosus, auricle, ventricle (base or apex), and the auriculo-ventricular ring, and also of skeletal muscle, smooth muscle, liver, and lung. The tissue for extraction was placed in a few cubic centimeters of Ringer's solution and left for periods varying from fifteen minutes to several hours, after which it was ground up in an agate mortar. The extract was allowed to settle and the clear fluid used for injection into the cannula.

In three cases the sinus extract caused definite augmentation and slight acceleration of the heart beat (fig. 5a), while in one the effect was so slight as to be uncertain. In another experiment the heart was absolutely quiescent when perfused with Ringer's solution only, but gave a few groups of beats when the extract of sinus was added. In the four remaining experiments, absolutely no effect was produced. No definite confirmation could be obtained of Haberlandt's contention (1924) that the stimulating substance is produced only in the beating sinus venosus, since in several cases where the extract was ineffective, the sinus had been beating in the solution for some time, and in other experiments where the extract caused marked augmentation no visible beat had been observed after separation from the rest of the heart.

It is important to note, moreover, that extracts of other parts of the heart behaved in exactly the same way, and that in every case where sinus extract had a stimulating action, extracts of auricle, auriculo-ventricular ring, base of ventricle, and even apex had an equal effect

(fig. 5a–d). In those cases where the sinus extract was ineffective, so also were extracts of every other region of the heart. This was true, not only for the myocardium, but also for other tissues. Extracts of skeletal muscle, smooth muscle, liver, and lung had a stimulating effect in about half the cases, the effects being exactly the same as those obtained with heart extracts (fig. 5e–h). If the sinus extract proved active, all other tissue extracts—whether heart or other tissue—also had a stimulating effect; if sinus extract were ineffective, then all tissue extracts tested were inert. No toxic effects were seen in any experiment on the frog heart.

Eutonon. In twelve experiments on the rabbit heart, injections were made of "eutonon,"[1] the "heart hormone" prepared from the liver by Prof. G. Zuelzer of Berlin (1928, also Zuelzer, Fisher and Müller, 1928). It was found that injection of about 0.006 gram produced a slight augmentation and acceleration, but this was always followed by a very definite, and occasionally fatal, toxic action (fig. 6). Larger amounts showed an increasing toxicity without any increased augmentor action while smaller quantities usually produced no effect. In some cases only a diminution of beat was produced, but no concentration could be found which produced a stimulating effect without subsequent toxic action.

Dried liver extract. The effect of an extract of ox-liver which had been partially purified and prepared as a powder by Dr. Michael Heidelberger for another investigation was also tested in three experiments. The liver extract in every case produced a slight augmentation, followed in one case by a definitely toxic effect. It was, however, much less toxic than Eutonon.

Histamine. The effect of histamine was found to be very similar to that of the various tissue extracts described, that is, an augmentation of varying intensity, with a less constant acceleration, often followed by a diminution of the beat, or complete stoppage.

Discussion of results. These results show without doubt that there is frequently present in extracts of heart muscle a substance which has a stimulating action upon the beat of the isolated heart, causing augmentation and often acceleration of the beat, and sometimes initiating a beat in a previously quiescent heart, but often followed by a toxic action.

The nature of this substance, however, appears less certain, as does also the question of its specificity and origin. According to Haberlandt (1927) it should be regarded as a true hormone, which is produced in the sino-auricular node and other parts of the "special musculature" of the heart, and has the specific function of initiating and controlling the heart beat. This viewpoint has been recently challenged by many other writers (Rigler

[1] I am indebted to Doctor Zuelzer for the opportunity to try his preparation "Eutonon" which was kindly brought to me from Europe by Dr. J. Marmorston-Gottesman.

and Singer, 1927; Rigler and Tieman, 1928; Rigler, 1928; Weichardt, 1927; Kisch, 1927); and the present research suggests that their doubts are well founded. There is no evidence that an extract of sino-auricular node is more active than that of other regions of the heart; in fact, an extract of the auricles (either right or left) usually has a rather more strongly augmenting action than that of the sino-auricular node. Other parts of the heart, also, are in many cases equally active, and the apex of the ventricle, completely free from endocardium to exclude the specialized conducting (Purkinje) cells, may have just as strong an augmenting action as the base.

In fact, in most experiments the effects of extracts of different parts of the heart are quite indistinguishable. No support, therefore, is obtained for the view that the stimulating substance is manufactured only in the special musculature, attractive though such a theory undoubtedly is.

It should also be remembered, as stated above, that this augmentation is by no means a constant effect; indeed, a toxic action is frequently obtained instead, as shown by a marked diminution or even stoppage of the heart beat. Such inhibition is often shown either as a preliminary to, or as an after-effect of, an augmentation, suggesting the possible existence of two substances in the extract, with opposite action on the heart muscle. The resultant effect depends upon the relative strength and rapidity of action of the two substances. The experiments show that both the augmentor and the inhibitor effects vary greatly according to the animal from which the tissues for extraction are taken. With rabbit extracts, augmentation is slight, inhibition is frequent; with cat, the augmentor effects are more marked, but so are the toxic after-effects, often fatally so. With extracts from sheep, the augmentor effects are very definite and the inhibition less marked, while with calf extracts augmentation is practically constant, and inhibition rare. With frog extracts tested on the frog heart, only augmentation is obtained, no inhibitor effects having been seen in any experiment.

If this augmentor substance is to be regarded as a specific stimulating hormone, it seems strange that the rabbit heart should respond least to extracts of rabbit heart, and should react so much more constantly and markedly to extracts from other animals.

It must be further noted that in order to obtain any augmentor effect relatively large amounts of extract, whether of sinus or other tissue, had to be used. For the rabbit heart, from 1 to 3 cc. of the extract corresponding to 0.1 to 0.3 gram of tissue were found to be necessary, even with active extracts, to produce augmentation. This by no means supports the idea of the presence of a specific stimulating hormone, but rather of a feebly acting substance present in var able amounts.

Moreover, a similar stimulating substance appears to be present in

many extra-cardiac tissues. Thus augmentation has been obtained with extracts of striped and smooth muscle from various organs, and also with extracts of liver and lung. The latter, in particular, shows an almost constant marked augmentor effect. The amount of the active substance is probably greater in the heart than in other tissues, but is undoubtedly not confined to the heart muscle. It was particularly noticeable in the frog heart, that if the preparation responded to extract of sinus, then it gave a similar augmentor response to injection of every other tissue extract —of skeletal muscle, smooth muscle, liver, and lung.

With regard to the action of liver extract, it is interesting to note the effect of "eutonon," the "heart hormone" prepared by Zuelzer (1928) from the liver. It was found to produce effects very similar to those of the other tissue extracts used in this research, except that the toxic (inhibitor) action was more marked than with most of the other tissues. With every injection, the augmentor effect, when marked or slight, was followed by a prolonged inhibition: no concentration could be found which would produce only an augmentation of beat.

Taking all these facts into consideration, it seems doubtful whether one is justified in regarding this augmentor substance as a specific hormone, whose function is to initiate and control the heart beat. The facts seem rather to suggest the presence of some product of metabolism in these tissue extracts. Weichardt (1927), indeed, has put forward the view that Haberlandt's "heart hormone" is merely a particular example of his general hypothesis of the "function-increasing" action of cleavage products.

Zwaardemaker (1928) has recently found a substance in skeletal muscle which stimulates the heart. He believes that this substance is present in an inactive form and suggests that activation may take place during life in the sino-auricular node.

Rigler and his co-workers (1927, 1928) have also found a stimulating substance in all parts of the heart as well as in other tissues. They suggest that the results may be explained by the presence in the extracts of histamine, which undoubtedly, as confirmed by these experiments, produces similar effects. It is perhaps of interest to remember in connection with the observation that lung extract has a marked and constant stimulating effect, that Dale and his collaborators (Best, Dale, Dudley and Thorpe, 1927) found a very high percentage of histamine in the ox-lung. According to their figures there is undoubtedly more than enough histamine present in the lung to account for the effects produced on the heart by the lung extracts.

Rigler and Tieman (1928) show that the effect of the "heart hormone" on the blood pressure and on the uterine wall is like that of histamine, and that both produce anaphylaxis-like effects in guinea-pigs. Likewise that the physical and chemical properties of this stimulating substance, so far

as is known, correspond with those of histamine. Haberlandt (1928), however, claims that in recent experiments he has obtained augmentation and acceleration with extracts free from histamine.

Frey and Kraut (1926; with Bauer, 1928) have found a substance in urine which has a stimulating action on the heart, increasing the amplitude of the beat and often causing acceleration of rhythm. These authors, however, do not believe (1926) that the active substance is identical with histamine, since they state that it will act in smaller concentration and does not cause the enlargement of the liver that is produced by histamine.

It appears certain, therefore, that not only is this heart-stimulating substance of somewhat inconstant action, and present in only small amounts, but that the special musculature of the heart is by no means the only tissue where it may be found. Whether ultimately it will prove identical with histamine or not, at present the evidence of its specificity as a heart hormone is extremely doubtful.

SUMMARY

1. The effect on the perfused rabbit heart of extracts of all parts of the heart is found to be an augmentation of varying degree, often preceded or followed by a diminution of beat.

2. This effect is somehwat inconstant and variable, but may be obtained from the extracts of the hearts of many different animals; e.g., calf extracts produce the greatest augmentation of the beat of the rabbit heart, and rabbit extracts the least.

3. Relatively large amounts of extract must be used to obtain this augmentation.

4. Similar effects, somewhat less marked, are obtained by the use of extracts of other tissues, such as striped and smooth muscle, liver, and lung.

5. Extract of frog heart, tested on the frog ventricle, produces augmentation in about 50 per cent of the cases, but has no inhibitor action. Extracts of other frog tissues have precisely similar effects.

6. The activity of the substance is unaffected by heat up to 100°C.

7. The effects of extracts of heart and other tissues are extremely like those of histamine.

8. The effect of "Eutonon," the "heart hormone" prepared by Zuelzer from the liver, is also very similar, but the after toxic action is more marked.

9. These results do not confirm the theory of a specific heart hormone, but rather support the view of a widely distributed substance occurring in most tissues, and possessing, in adequate concentration, an augmenting action on the heart.

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CONTROL OF CAPILLARIES OF SKELETAL MUSCLE[1]

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In spite of much work that has been done in recent years on the control of the capillary circulation the question is still unsettled.

Hooker reviewed the subject several years ago and came to the conclusion that "we have a considerable mass of evidence indicating that the behavior of these vessels must be of very great functional significance." The capillary bed is responsive to both chemical and nervous influences. Hooker said that "Broadly speaking, we may say that chemical factors, so far as they have been studied, mediate dilatation of the capillaries and venules, while nerve stimulation mediates constriction of these vessels."

Krogh brought together the evidence for independent contractility of the capillaries. He pointed out the fallacy of concluding that a capillary is contracted if the corpuscles disappear. The precapillary vessel may contract so that corpuscles cannot pass although the plasma flows and the capillary may be open. To make certain the capillary walls must be observed.

Steinach and Kahn were able by electrical stimulation to produce contraction of capillaries in excised membranes of the frog and in the omentum of the cat.

Many have observed the changes which occur in human skin and have concluded both from direct observation and inference that the capillaries act independently of the other vessels. Ebbecke called attention to the blood which fills the congested cutaneous vessels when the hand is exposed to cold. He concluded that the capillaries were dilated while the arterioles were constricted so that the reduced blood flow carried insufficient oxygen for the needs of the skin accounting for the blue blood. Blood may flow through the hand rapidly enough to make it warm, yet it may be pale. He concluded that the arterioles were dilated more than usual without much change in the capillaries.

Carrier studied the circulation in the human skin by the use of an arc light. At 25°C. she found all of the capillaries open. Below 20°C. there was contraction and relaxation of the capillaries and disappearance of the

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arterioles. When the skin became blue with cold, the capillaries were filled with blood. She was able to cause constriction of the capillaries by injecting a strong solution of adrenalin (1:1000 to 1:10,000) beside the capillary.

Krogh observed the superficial capillaries in the muscles of the frog and guinea pig, using strong reflected light. Resting muscle was pale, only a few capillaries being seen at fairly regular intervals. The corpuscles passed through slowly. If the muscle was caused to contract a large number of capillaries could be seen. These were dilated and carried blood at a rapid rate. Some minutes after the contractions had ceased, the capillaries became narrower and most of them became empty. Exposure to air and strong light always increased the circulation.

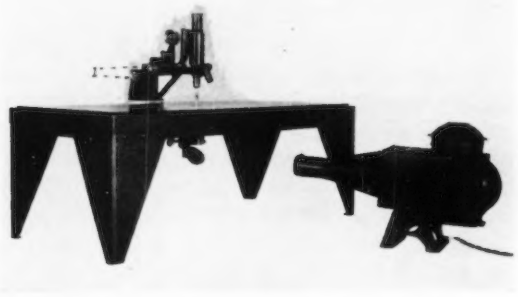

Fig. 1

Krogh also injected India ink into the circulation of living animals and suddenly killed them by stopping the circulation. The tissues were then fixed and sectioned. Open capillaries were detected by the presence of particles of ink. According to this method he found few capillaries open in the resting muscle but muscles that had been tetanized before the ink injection contained a large number of open capillaries.

Nesterow came to the conclusion that the great factor in the variation of the capillary circulation is the precapillary arteriole.

We have now studied the skeletal muscle of more than one hundred living cats by the use of transmitted light. This is a report of our observations on the capillaries.

APPARATUS AND METHODS OF OBSERVATION. In the earlier work included in this report, we employed an apparatus already described (Hartman, Evans and Walker). More recently we have used an apparatus made for us by the Spencer Lens Company of Buffalo. It consists of an ordinary

compound microscope mounted on a very rigid support extending over a small table (fig. 1). The microscope can be moved in a horizontal plane in both directions by means of fine adjusting screws (1). The light is reflected through a condenser into a glass rod which extends upward through a hole in the table. The height of the rod is adjustable. The tissue to be studied is placed on top of the rod so that illumination is by transmitted light.

The animal was fastened on a small animal board which contained holes under each thigh. A slit was made in the skin of the thigh above and below in the region of the sartorius muscle which was to be studied. The board was then placed in position and clamped. Then the glass rod was run up under the sartorius, between the muscles lying below, care being taken that there was no intervening fat or connective tissue. The connective tissue was carefully removed from the sartorius above, Ringer's solution was applied and a small cover glass put in place to prevent drying. The height of the glass rod was adjusted so that the muscle was not stretched and the circulation thereby impeded. With good preparations it was possible to study the circulation under the oil immersion lens (1.8 mm.) although usually the 4 mm. objective was employed. Sometimes the 16 mm. objective was used. The 10 X ocular was employed in most of the work. When measurements were made the ocular micrometer was used. A four hundred watt Mazda lamp was used ordinarily although a small arc light filtered through a solution of picric acid and copper sulphate was sometimes employed.

In our earlier work, the criterion of open capillaries was the presence of corpuscles, later the capillary walls were observed when in doubt. The effect of anesthetics on the capillary reaction was ruled out as far as possible by using different anesthetics; ether, chloralose, and urethane, or decerebration. When no anesthetic is mentioned ether was used. All observations were made on cats. Special methods will be described in their appropriate places.

It will also be convenient to discuss the investigations of others in relation to our own observations in each section rather than at the conclusion of the paper.

India ink injections. India ink (F. Weber, Philadelphia) was prepared for injection by dialyzing many hours against Ringer's solution through a thin collodion sac to remove deleterious substances. It was then filtered through no. 5 Whatman paper to remove large particles. The prepared ink was sometimes diluted by an equal volume of distilled water.

Injections into the general circulation were unsatisfactory because the ink was too dilute by the time it reached the part to be studied or the particles were removed from the circulation by the lungs or other tissues. The

method finally adopted was to inject into the femoral artery through a fine needle. Direct observations of the live muscle (tibialis anticus) after ink injections were made in nine cats.

One might expect the capillaries to be flooded with ink particles after injection because the muscle appeared black. But this was not the case.

In thirty seconds or longer after the beginning of the injection a few particles of ink could be seen in the capillaries, but they soon disappeared. At times some of the open capillaries were devoid of ink particles. Upon investigation it was found that clumps of particles were caught at the junction of the arteriole and capillaries so that they did not enter the latter.

After many attempts we decided that the ink injection method did not give a true picture of the open capillaries.

NERVE STIMULATION. *Femoral.* The femoral nerve was directly stimulated at the inguinal ligament by an induced current using rapidly repeated shocks.

In an animal under urethane anesthesia 15 seconds' stimulation (coil at 350 mm.) produced a decrease in flow and disappearance of capillaries. After the stimulation stopped there was dilatation, the venules especially were dilated and full of blood. The striations of the muscle appeared and the field became brighter. These results were obtained repeatedly in this animal. Two other cats were studied similarly. They did not show constriction of the capillaries.

In order to rule out reflex effects the femoral nerve was cut in three animals a few minutes before the experiment started. The cutting of the nerve not only produced characteristic stimulatory effects but the dilatation so caused did not entirely disappear during the course of the experiment. The effects described in table 1 are typical of other experiments.

The usual effect of stimulating the femoral nerve is dilatation of the capillaries. Arteries and veins constrict if the stimulation is prolonged.

Sympathetic. Observations upon skeletal muscle with stimulation of the sympathetic have already been published (Hartman, Evans, Malachowski and Michalek). The typical effect is dilatation of capillaries and increase in the rate of blood flow. The field usually becomes clearer and the striations more distinct. There also develops a peculiar transverse vibration of the muscle fibers.

One animal reacted somewhat differently. This cat, however, was not normal. The thyroid had been removed two months before and an open infection developed. The right adrenal and the testes were removed two weeks before. At the first operation the cat weighed 3.65 kgm. At the time of the experiment it weighed only 2.64 kgm. We cannot be sure that the capillaries constricted. They disappeared from view. Table 2 gives observations on this animal.

REFLEX EFFECTS. Reflex effects upon the sartorius were studied by

TABLE 1

Stimulation of femoral nerve with induced current

Cat, 1.76 kgm. Ether

DISTANCE OF SECONDARY COIL FROM PRIMARY	DURATION OF STIMULATION	RESPONSE IN SARTORIUS
mm.		
Femoral nerve cut		Field, which was opaque, became bright, all vessels dilated, continuous twitching
350		During stimulation field became very bright, striations appeared and blood flow improved. Twitching, jerky in nature, was present
300	20 seconds	Marked increase in blood flow, capillaries dilated. Continuous transverse twitching. Field very bright
250	7 minutes	All capillaries open and dilated. Most capillaries 7.35μ. Some arteries and veins constricted by beading
200	25 minutes	Detailed observations impossible during stimulation because the muscle fibers vibrated too strongly. The field was very bright and the blood flow very fast
After stimulation		All capillaries open and dilated. Some veins beaded
	3 minutes	Field not so bright
	6 minutes	No twitching
	7.5 minutes	Now and then adrenalin-like twitching when field became extremely bright

TABLE 2

Effect of sympathetic stimulation

Cat with thyroids, testes and one adrenal removed

DISTANCE OF SECONDARY COIL FROM PRIMARY	DURATION OF STIMULATION	RESPONSE IN SARTORIUS
mm.	*seconds*	
350	15	Slight dilatation of vein and venule, slower blood flow
300	20	Vein constricted (23.0 to 17.7μ). Slower flow. Precapillary arteriole dilated. Venule constricts and dilates alternately after stimulation
200	20	Precapillary arteriole and capillaries dilate after ten seconds' stimulation
100	20	Capillary dilates at 30 seconds, flow slower. Then capillaries appear to constrict (70 seconds)
50	20	Capillaries dilate and then constrict
0	20	Capillaries constrict in 10 seconds

stimulating the shaven skin of the abdomen. Electrical shocks, heat and cold were used as stimuli. The observations reported here were upon a cat anesthetized with urethane.

Electrical. The skin, previously soaked with salt solution, was stimulated with a tetanizing current. With the secondary coil 100 mm. from the primary, stimulation for 15 seconds caused an increase in the velocity of the blood flow and slight dilatation of the capillaries. The field also became clearer.

Cold. Ice applied to the abdomen for one minute caused dilatation of the capillaries and venules and an increased blood flow in the sartorius muscle. An arteriole dilated. Circulation in a vein at first stopped completely, then the vein dilated and the flow increased. The field became more opaque and some twitching occurred.

Heat. A wad of paper toweling soaked in hot water was applied to the abdomen for one minute. The blood flow in the sartorius increased. Capillaries, venules, arterioles, an artery, and a vein dilated. The field became clearer. Ice and hot packs were applied alternately and repeatedly. Although ice caused dilatation, heat increased the dilatation which had not entirely disappeared from the effects of ice.

SHOCK. It has been shown by Cannon and others that the red corpuscles accumulate in the capillaries in surgical shock.

We have been able to observe the capillaries of the sartorius while shock was being induced. A small opening was made in the duodenum. Forceps were inserted through this into the pylorus where they were turned about, stretching the pylorus. A few minutes stimulation caused a fall in blood pressure and the development of the symptoms of shock.

In one cat, six minutes after pyloric stimulation had been in operation, the circulation had stopped almost everywhere in the sartorius. The capillaries were dilated and filled with red corpuscles collected in groups. The field became hazier and the cross striations of the muscle fibers less distinct. Two minutes after the stimulation had ceased the blood flow, through the capillaries, returned. The capillaries were still dilated. Three minutes' stimulation of the pylorus again caused the blood flow in most of the capillaries to cease.

INFLUENCE OF VARIOUS SUBSTANCES. In a few of the early experiments dilute solutions of a number of substances were applied locally to the sartorius. The results are of little importance because the concentration was so great that the tissue was usually damaged. Their effects will be briefly described.

Acids. Dilute *acetic acid* applied and then washed away after 5 seconds caused the circulation to stop in the smaller vessels. All vessels appeared constricted. The field became more opaque, The vessels seemed to be permanently injured. Only one preparation was tested.

Acetic acid (1:1000) was injected intravenously into a cat weighing 1.57 kgm. One-half cubic centimeter caused an increase in the blood flow (sartorius) and dilatation of the capillaries and of the pre- and post-capil-

lary vessels in 55 seconds. A venule dilated from 7.35 to 9.8μ. No change in the caliber of the arteries and veins could be seen. One cubic centimeter caused dilatation of a venule from 24.5 to 34.3μ, a vein from 88 to 97μ and an artery from 26.6 to 35.4μ. The capillaries and contiguous vessels were dilated.

In order to test the effect of a stronger acid, *hydrochloric acid* was injected into three cats in doses ranging from 1 cc., 1:1000 to 1 cc., 1:100. Capillaries and other small vessels were dilated and the circulation was increased. In two animals there was violent longitudinal twitching of the muscle fibers. One cubic centimerer 1:100 caused dilatation of a venule from 14.7 to 24.5μ. The capillary dilatation for this dose lasted for six minutes.

Acetyl choline. We have not made a very thorough study of the action of acetyl choline but inasmuch as our results were all concordant they are of value. The changes produced by acetyl choline hydrobromide were studied in five cats. In three a 0.3 per cent solution was applied locally. The animals were young, weighing 0.75 kgm. There was dilatation of all the visible vessels, i.e., arterioles, capillaries and venules. The circulation became slower. The field became cloudier and twitched continually in one animal.

Acetyl choline was administered intravenously in the other two cats with similar results, dilatation of arterioles, venules and capillaries. Injection of 0.2 cc. of a 5 per cent solution (into a 1 kgm. cat) produced extreme dilatation of all vessels in 15 seconds.

Acetyl choline gave the most marked dilatation of all drugs used.

Dale and Laidlaw believed that acetyl choline dilated the arterioles only. Our results include capillaries and venules in the dilatation.

Amyl nitrite. Amyl nitrite was administered by inhalation through a tracheal cannula to six cats, four decerebrated and two etherized. It produced such marked dilatation in all vessels, especially the capillaries, that the circulation stopped. The following experiment is typical:

Amyl nitrite was administered for twenty-eight seconds to a cat weighing 2.2 kgm. In 10 seconds from the beginning of administration an arteriole had dilated from 26.4 to 35.2μ. In 20 seconds a vein had increased from 52.8 to 61.6μ. In 80 seconds all capillaries were dilated while in 100 seconds the arterioles and venules were dilated as well. In 115 seconds the circulation had stopped. The field became bright and the striations of the muscle fibers clear. In 145 seconds the circulation was resumed.

Atropine. Atropine sulphate (1:1500 solution) was injected intravenously into five cats.

The typical effect was shown in a cat weighing 1.45 kgm. Injection of 0.6 cc. caused dilatation of the capillaries, venules, arterioles and a vein. New capillaries also appeared. A vein dilated from 26.5 to 35.4μ four minutes after injection. One cubic centimeter caused such great dilatation everywhere that the circulation in the sartorius stopped temporarily.

A kitten 14 weeks of age (0.8 kgm. weight) had the right femoral nerve cut eight weeks before the experiment. The right sartorius was used for study.

One cubic centimeter caused a small dilatation of the capillaries but this was observed quite late, seven minutes after the injection.

A vein and an artery dilated periodically as follows:

TIME AFTER INJECTION		VEIN	ARTERY
minutes	*seconds*	μ	μ
Normal		70.8	44.0
1	30	88.5	79.2
3		70.8	
4	10	88.5	70.8
5	30		79.2
6		106.2	88.5

Calcium chloride. A 5 per cent solution of calcium chloride was injected into the jugular vein of a 1.3 kgm. cat. One-half to 1 cc. caused an increase in the rate of flow, a beading of venules and of an arteriole with dilatation of the capillaries. One capillary dilated from 4.9 to 8.8μ.

One cubic centimeter caused distinct dilatation of capillaries and slowing of the circulation which was stopped in some capillaries. The larger vessels seemed to be distinctly constricted. The veins and arteries, especially the former, were beaded.

Cocaine. Cocaine hydrochlorate (1.5 per cent) was injected intravenously into two cats weighing 0.95 and 1.45 kgm. In the first animal 1 cc. caused extreme dilatation and cessation of the circulation. A vein dilated more than an artery at 45 seconds. But at 135 seconds an artery constricted. At 3 minutes the circulation started again and breathing, which had stopped, was resumed. The results were similar in the second animal except that the flow stopped for a shorter period and respiration was not interrupted.

Local application of cocaine to the sartorius muscle caused dilatation of all vessels in a third animal.

Cocaine, therefore, seems to produce dilatation of capillaries, venules, arterioles and sometimes larger vessels.

Ephedrine. Ephedrine sulphate (Lilly) was compared with adrenalin in two cats. A solution (0.12 per cent) was injected intravenously into a cat weighing 1.4 kgm. Dilatation of capillaries, arterioles and venules resulted. Injection of 0.4 cc. caused a vein to dilate from 8.8 to 10.6μ and an arteriole from 4.6 to 5.3μ. Dilatation of the capillaries was not measured.

Eight-tenths of a cubic centimeter caused a vein to dilate from 15.6 to 16.9μ, an arteriole from 4.6 to 6.2μ and a venule from 6.2 to 7.8μ.

The capillaries dilated, the field became cloudy and the blood flow improved.

A solution of 0.3 per cent was injected intravenously into a cat of 2.2 kgm. One and five-tenths of a cubic centimeter caused dilatation of the capillaries but slight constriction of the larger vessels.

Capillary dilatation from ephedrine is not nearly so marked as from adrenalin, nor do twitching and brightening of the field occur as with the latter substance.

Ephedrine produces a rise in blood pressure due to vaso-constriction and cardiac stimulation. It is much less powerful than epinephrin but its effects persist longer (Pittenger). According to our observations, the constriction occurs in the larger vessels; capillaries, venules and arterioles dilating. Ephedrine makes the field cloudy, epinephrin brighter.

Epinephrin. The effect of epinephrin has been reported (Hartman, Evans and Walker). Doses ranging from 0.2 cc., 1:100,000 to 1 cc., 1:10,000 were injected into the general circulation.

Small doses caused dilatation of the capillaries, venules, arterioles, veins and arteries in skeletal muscle. The blood flow increased. Later the flow might decrease. With larger doses (1 cc., 1:100,000 or above) the capillaries and very small vessels dilated as before while the veins, arteries and arterioles constricted. Epinephrin made the field clearer and brighter and sometimes caused twitching or vibration of the muscle.

In the present study we tried the effect of greater concentrations of epinephrin by local application. It was with such concentrations that Carrier obtained capillary constriction in the skin.

When the local concentration of adrenalin became sufficiently high, the capillaries constricted. Thus application of a 1:10,000 solution caused constriction or disappearance of capillaries in 26 seconds. At the end of one minute the field was becoming brighter and more capillaries were disappearing. In 2 minutes and 15 seconds the capillaries were reappearing. In 4 minutes all capillaries seemed to have reappeared and the field seemed normal. A second application of adrenalin, some time after histamine had been applied locally and the effects apparently worn off, produced results similar to the first. The constriction of the capillaries, however, was not so marked. Characteristic adrenalin twitching also appeared.

Similar changes were produced in a second animal. After the typical reaction to the local application of adrenalin in this cat, cocaine was applied locally. All vessels dilated. Three minutes after the application of cocaine 1:10,000 adrenalin was applied. A brighter field and adrenalin twitching were produced but constriction of the capillaries was very slight if present at all. Two additional applications of adrenalin gave the same results.

Ergotamine. Ergotamine tartrate "Sandoz" was injected intravenously into two cats (1 cc. contained 0.0005 gram ergotamine tartrate). Records of blood pressure in a cat weighing 2.1 kgm. were taken during the experiments. One-tenth cubic centimeter ergotamine solution caused a slowing of the circulation with dilatation of capillaries and stopping of the flow in some. Arterioles seemed to have dilated. The effects on blood pressure with this dose were inconstant giving a fall the first time, and a fall and then a rise with the second injection. The field became brighter and then very dark with some injections.

Ether. Ether was applied locally in one preparation. It caused dilatation of the blood vessels and clouding of the field. This effect has also been noted in ether anesthesia (Hartman, Evans and Walker).

Ethyl alcohol. Ethyl alcohol was studied only by direct application of a dilute solution. The results obtained have little significance because the amount of alcohol reaching the capillaries was much larger than any that could reach the capillaries through the circulation. The capillaries seemed to be completely constricted 25 seconds after the application. The arterioles were constricted in 35 seconds. At 40 seconds the circulation had stopped in all but a few places where it was quite sluggish. At 100 seconds the vessels had dilated and the circulation was returning to normal. At 2 minutes and 45 seconds the whole mass of muscle fibers fibrillated. The field had become normal in 5 minutes.

Ferric chloride. A 2 per cent solution of ferric chloride was applied to the sartorius in three cats. In one all vessels appeared to be constricted and injured. Blood flow ceased. In the other two, dilatation of the vessels occurred followed by permanent damage.

Histamine. Numerous workers have studied the effect of histamine on the circulation. Dale and Richards concluded that histamine caused relaxation of the tone of the capillaries and constriction of arteries. Inchley (1923) by perfusion methods appeared to be able to show that both arterioles and venules constrict under the influence of histamine. He believed (1926) that histamine shock was caused by occlusion of the great veins so that permanent relaxation of the capillaries followed. Carrier found that histamine (1:10,000 to 1:1000) applied at the base of the nail in the human caused dilatation of the capillaries.

We have studied the effects of histamine in our preparation of the sartorius muscle in ten cats. Examples of these are described.

In only one animal, a kitten seven weeks old, did we try the direct application of histamine (1:10,000). One minute after application the circulation stopped. Ninety-seven seconds after application many vessels were dilated. Blood was oscillating back and forth in the large vessels.

Our other observations were made after intravenous injections. Study of the sartorius of a large cat (3.5 kgm.) was made with a magnification of

100 diameters. The changes are described in table 3. Capillary walls were not visible under the magnification so that the only indicator of open capillaries was the presence of corpuscles in them. Briefly a slower blood flow was followed by disappearance of all capillaries. Then the capillaries began to appear again. The blood flow increased. More capillaries appeared and others dilated. Dilatation later was accompanied by retardation of the circulation. Finally all vessels appeared to be dilated and the flow was very slow.

In another cat (1.4 kgm.) these observations were confirmed. The effect of the histamine seemed to disappear in ten to eleven minutes even with doses as large as 1.25 cc., 1:10,000. In these experiments, whatever the

TABLE 3

Effect of injecting 1 cc., 1:10,000 histamine in a cat (3.5 kgm.)

TIME AFTER INJECTION		RESPONSE IN SARTORIUS—LOW POWER (100 DIA.)
minutes	*seconds*	
	45	Flow of blood much slower
1		Flow almost stopped in a small venule
1	25	All capillaries disappeared, i.e., no corpuscles present for their identification
2	42	One capillary appeared, flow slow
3	7	Flow faster in small venule
3	20	Flow much faster throughout. New capillaries appeared
3	40	Slight dilatation of small venule
4		More capillaries opened up during the next 103 seconds
5	43	Capillaries slightly dilated
6	25	Capillaries dilating, flow slightly slower
6	55	Capillaries further dilated, flow slower
7	15	A new capillary appeared
7	55	A new capillary appeared
8	35	Increase in rate of flow in capillaries and venules
9	5	Flow in capillaries very slow
12		All vessels very much dilated, flow very slow

dose, there was a certain period shortly after the injection in which no vessels could be distinguished, under low power. At first it appeared to be due to a change of focus in the field. This was not the case; therefore, it must have been due to constriction somewhere.

In another old cat (3.51 kgm.) the effect of 1 cc., 1:10,000 histamine did not disappear until nineteen minutes had elapsed.

A more dilute solution of histamine, 1:100,000, was used in a small cat (1.3 kgm.) and observations were made with high power (440 dia.).

One-half of a cubic centimeter of this solution caused dilatation in the capillaries and venules and beading of an arteriole. The blood flow stopped. These conditions developed within two minutes.

One cubic centimeter produced similar but more marked effects. Arterioles again showed alternate bands of constriction or beading. A very large vein (low power) seemed to be constricted. Some capillaries were twice the diameter of others. The venules were dilated. Pre-capillary arterioles appeared somewhat dilated although not so much as the post-capillary venules.

The effect of histamine was tried on a sartorius whose nerve (femoral) had been cut 50 days before. Four-tenths cubic centimeter of 1:10,000 histamine given intravenously (cat weighed 1.35 kgm.) caused the following changes as seen under the low power.

Time *sec.*	
25–30	Dilatation of venules and much brighter field.
40	Distinct dilatation of capillaries.
45	Greater dilatation of venules. One venule dilated to twice the original diameter (9.8 to 19.6μ).
85	All capillaries in field dilated.
95	Venules continue to dilate.
170	Arterioles very much dilated (observations discontinued because ether was given).

Twice the dose of histamine produced similar but more pronounced results.

This was the only denervated muscle observed in connection with histamine. It will be noted that the arterioles dilated. In normal muscle they seemed to dilate little or not at all. Indeed, they might even constrict.

Aside from the effect on blood vessels histamine usually produced a brighter field and in three animals it produced slight continuous twitching of the muscle fibers.

Our observations with histamine on blood vessels of the sartorius muscle of the cat may be briefly summarized thus: With small doses there was dilatation of capillaries and venules, with larger doses the capillaries and venules dilated while the arterioles, arteries and veins constricted.

Hydroxides of Ammonium, Potassium and Sodium. Dilute ammonium hydroxide was applied to the sartorius of a kitten weighing 0.75 kgm. Capillaries and venules appeared to constrict and then to dilate, the capillaries dilating more than the other vessels. Similar application in a second kitten of about the same age caused new capillaries to appear (30 sec.), slowing of the circulation and finally a temporary cessation of flow (130 sec.). At 3 minutes the blood began to flow. All vessels including capillaries were dilated. Hemorrhage suddenly began to appear (4 min.) in various sections of the field.

In a third cat (1.57 kgm.) 1:10,000 ammonium hydroxide was injected intravenously.

One cc. caused slower flow in the capillaries, post- and pre-capillary vessels (45 sec.). The capillaries became dilated. The post- and pre-capillary vessels were slightly dilated (100 sec.). An artery constricted from 26.5 to 17.7 μ. A vein constricted from 53.1 to 49.5 μ.

Two cc. in 30 sec. caused changes shown in table 4.

One-half cc. potassium hydroxide (1:10,000) injected intravenously into the same cat produced fine fibrillation and a hazy field. One cc. produced slight dilatation of capillaries and small venules. One and one-half cc. acted like ammonium hydroxide causing dilatation of capillaries and of pre-capillary and post-capillary vessels. However, one arteriole constricted from 35.4 to 26.5 μ and one venule from 79.6 to 62.0 μ. Many new capillaries opened. The field was hazy but bright and microscopic fibrillation was present.

TABLE 4

Effect of 2 cc., 1:10,000 NH_4OH injected intravenously

Cat 1.57 kgm.

TIME		RESPONSE IN SARTORIUS
minutes	*seconds*	
	20	Blood flow slow everywhere
	40	Capillaries dilated from 4.9 to 6.4μ
1	5	Post-capillary venules dilated from 5.9 to 7.35μ
1	25	Field hazy. Circulation stopped in some capillaries
1	45	Some capillaries appeared beaded although dilated
2	15	Good flow in capillaries
2	25	Artery constricted from 26.5 to 19.5μ
3	20	Vein constricted from 53.1 to 49.5μ
		Ether given, observations discontinued

A one to two thousand solution of sodium hydroxide produced effects similar to ammonium and potassium hydroxides—dilatation in capillaries and usually in pre-capillary vessels. The larger vessels, however, with a dose of 1½ cc. constricted, a vein from 88.5 to 70.8μ and an arteriole 26.5 to 21.2μ.

Liver extract. The effect of heparmone B (Lilly) was observed in one animal. From 0.2 to 1 cc. was injected. General dilatation was produced and the circulation was stopped everywhere except in limited areas. In 4.5 minutes, the capillaries were extremely dilated. The heart became slow and weak. Muscle fibers twitched slightly. The smaller dose produced only a small dilatation.

Nicotine. Local application of 1:2,000 solution of nicotine caused at first a small dilatation with a slower circulation and finally a marked dilatation of the capillaries.

The injection of 0.3 cc. of a 1:2,000 solution of nicotine into the jugular

vein of an eight weeks old kitten (950 grams) caused at first constriction in an artery (15 sec.) and later (75 sec.) dilatation in the same artery. This artery was still dilated after 6 minutes. Capillaries were not observed with this dose but with 0.8 cc. all capillaries dilated, the field became hazy and no striations could be seen. This haziness was greater than that caused by ether.

In another cat (1000 grams weight) 1.5 cc. of the same concentration caused, in forty seconds, great dilatation of all vessels observed. At 70 seconds veins showed "peristaltic" movement.

Picrotoxin. Picrotoxin (1:15,000) was injected intravenously into two kittens. One-half of a cubic centimeter injected into a kitten weighing 1 kgm. caused dilatation of capillaries, venules and small arterioles. One of these arterioles was constricted at the end coming from the artery and dilated at the end joining the capillary. Very fine twitching, almost like that produced by adrenalin, was observed. Circulation became slow and blood escaped from the vessels. Picrotoxin appeared to produce permanent damage in this animal. In the second animal which weighed 0.95 kgm., 0.2 cc. picrotoxin solution produced the twitching already described. No other changes were noted. With each injection of picrotoxin the animal seemed to go into slight shock, the shock increasing with increased dosage. One cubic centimeter injected into this animal caused, in 3 minutes, marked dilatation of capillaries and some dilatation of all other vessels, venules being more dilated than the arterioles.

Pituitrin. Pituitrin was injected into two animals. One-half of a cubic centimeter of 1:10 was administered in each instance. In a cat weighing 1.75 kgm. the changes in the sartorius were similar to those produced by adrenalin, the smallest vessels dilated and the largest vessels constricted. Injection into the second animal produced like results in the small vessels, the larger vessels not being observed.

Urethane. A 25 per cent solution of urethane was applied to the sartorius in three cats anesthetized with ether. There was great retardation, sometimes cessation, of blood flow. The vessels were markedly dilated. In one animal new capillaries opened and the field became slightly cloudy.

Effect of fatigue products. The effect of fatigue products has been studied by stimulating the peripheral cut end of the brachial plexus and observing the changes in the sartorius muscle. Lactic acid and carbon dioxide have also been administered. These results have already been reported (Hartman, Evans and Malachowski). Epinephrin seems to be released, producing its effects q. v. Independent of epinephrin, other products formed cause dilatation of the capillaries and produce a hazier field. Release of lactic acid could account for the latter. Carbon dioxide produces similar effects. These effects are not modified by cutting the sympathetic nerve fibers.

Effect of hemorrhage. Pilcher and Sollmann have shown that the withdrawal of blood from dogs progressively stimulated, depressed, and finally paralyzed the vasomotor center. Such an effect resulted in a slight general constriction, and then great dilatation of the peripheral vessels. Meek and Eyster observed the venules and capillaries in the ears of dogs before, during and after hemorrhage. After removing 100 cc. of blood (about 2.7 per cent of the body weight) from the femoral artery the blood vessels in the ears slowly decreased in diameter. In another dog withdrawal of 200 cc. of blood resulted in "almost instantaneous whitening of the ear

TABLE 5

A cat weighing 2.65 kgm. (anesthetized with urethane) was bled from the carotid artery at a uniform but slow rate (50 cc. in 17 minutes) with the following results:

TIME		BLOOD PRESSURE	RESPONSE IN SARTORIUS
minutes	*seconds*	*mm.* Hg	
Start		130	
2	25	104	Venule dilated
3	30	98	Capillaries dilated; new capillaries opened
4	20	102	Some cells in inner wall of venules became bright and then dark
5	55	106	Muscle striations very pronounced
6	20	106	More capillaries continued to open
7		100	Capillaries so dilated that the corpuscles could pass three abreast
8		97	Flow sluggish
9		81	Corpuscles scattered in all vessels
13		54	Circulation very sluggish; stopped in many capillaries
17	55	32*	Muscle fibers darker; striations less distinct. Corpuscles very scattered
20	(Bleeding stopped: 3 minutes)		Circulation renewed in some vessels
23	5	(10 cc. more removed)	Circulation stopped entirely. Heart beating poorly. All vessels in the field extremely dilated

* Total bleeding is 50 cc.

caused by the constriction of the venules and capillaries." These investigators used Hooker's method of illumination (1) a method which, according to Hooker himself, would not give a definite criterion for changes in diameter of capillaries.

Our observations on the sartorius muscle tend to show that in no stage of bleeding is there constriction of capillaries or venules. In fact, they respond to bleeding by dilatation. Since arterioles dilate also, one must look elsewhere for the peripheral vasoconstriction so often reported. Perhaps the larger vessels constrict. Penfield obtained constriction of the perfused vessels in the hind leg of the dog after hemorrhage.

The compensatory constriction in small hemorrhages may be accounted for in part by the shifting of blood from organs like the spleen and liver into the general circulation.

Barcroft and others found that the spleen contracts during hemorrhage so that enough blood is delivered into the general circulation to almost compensate for the loss at first. The liver likewise gives up blood during hemorrhage (F. R. Griffith Jr.[2]).

The results of slow bleeding in a cat are shown in table 5.

TABLE 6

Cat, 1.25 kgm. Ether. Sartorius observed under both low and high powers so that the larger vessels could be studied. Ether discontinued during observations.

BLOOD REMOVED	TIME	RESPONSE IN SARTORIUS
cc.	*a.m.*	
5	10:59	Capillaries dilated. Field became brighter. Muscle twitched. Within three minutes the field again cloudy and twitching disappeared
5	11:02	Dilatation of capillaries and brighter field
5*	11:04	As above
5	11:07	As above except that twitching developed
5	11:13	Apparently slight constriction in arterioles and venules
5	11:15	Apparently slight constriction in larger vessels. Striations clearer. Blood cells flowing in groups with gaps of plasma between
5	11:18	Field brighter; occasional twitching. Circulation became slower everywhere and then entirely stopped
	11:23	Circulation started, cat gasping. Field very cloudy (no ether given since 10:50 a.m.)
	11:30	Field extremely bright; respiration stopped. Circulation very good but all vessels dilated. No great dilution of blood

* More rapidly.

In addition to dilatation of the vessels the early dilution of the blood should be noted (6 to 7 minutes). At first (about six minutes) the muscle fibers become clearer and later (about 18 minutes; 50 cc. bled) less distinct.

In six cats the quantity of blood removed was measured but the blood pressure was not taken. These cats ranged in weight from 1.25 to 3.1 kgm.

Rapid removal of 10 cc. or less from the carotid artery caused dilatation of the capillaries and a slower blood flow. Some arterioles dilated and new capillaries opened. Bleeding was stopped for a few minutes (3 to 8 minutes) following the removal of each 10 cc. portion. It was found that the capillary flow recovered between hemorrhages.

[2] Unpublished results.

The results from one of these animals is shown in table 6.

Arterioles were not studied in the two cats reported in these tables but in others these vessels have been observed to dilate as a result of hemorrhage.

TABLE 7

Effect of injecting Tyrode's solution intravenously

Cat 3.6 kgm. (Urethane 2 grams per kilo)

TYRODE'S SOLUTION INJECTED	BLOOD PRESSURE IN CAROTID	RESPONSE IN SARTORIUS
cc.	*mm.* Hg	
20*	117 at start 125	Increased rate and volume of flow, vessels dilating but no new capillaries appeared. Dilated capillaries soon began to constrict and circulation returned to normal
20†	117 130	As above except that dilution was so great that red cells were wide apart. Two new capillaries appeared filled with plasma
20†	112 112	Great dilution of corpuscles, new capillaries appeared, rate and volume flow increased
20	124	Further dilatation of capillaries
20	120 124	More new capillaries opened
20	126	11 seconds after injection started, great increase in rate of flow; dilatation 27 seconds after injection started, new capillaries appeared 40 seconds, red cells spherical 76 seconds, only an occasional corpuscle seen in the fluid passing through the capillaries 85 seconds, capillaries begin to constrict 105 seconds, two capillaries disappear. Some capillaries still about twice normal diameter
20	104 113	As before also field very clear including muscle striations

* In 75 seconds.

† In 90 seconds.

Effect of infusion. According to Meek and Eyster the increase in the volume of fluid, by the intravenous injections of physiological salt solution, gum-saline or blood, ranging from 25 per cent to 103 per cent of the original blood volume, is soon taken care of by the capillaries and venules which act as reservoirs. We have studied the circulatory changes in the sartorius muscle of the cat following intravenous infusions.

Injections of Locke's solution were made intravenously into three normal cats, one under the influence of urethane and the others under the influence of ether. With small quantities (2 or 3 cc.) the only noticeable effect was the increased speed of the circulation and the dilution of the corpuscles. Sometimes the field appeared brighter.

Larger quantities of solution produced very definite effects as illustrated in table 7. Twenty cubic centimeter quantities (cat weighed 3.6 kgm.) were injected over periods of 60 to 90 seconds long. Just sufficient time intervened between the injections so that the heart was not overtaxed. Carotid blood pressure was recorded. In a few seconds, about ten, after an injection started the circulation increased in speed. In about thirty seconds the capillaries showed definite dilatation. This dilatation began to disappear soon after the injection ceased and the circulation returned to normal. After the second and succeeding injections the blood was so dilute that the red cells were widely separated. Frequently new capillaries appeared in the field to disappear as the injection effects wore off. The capillaries often dilated to twice the original size. The field became very clear and the striations of the muscle fibers very distinct. Injections were repeated until a total of 300 cc. had been given. After the first three or four injections the succeeding injections produced merely a repetition of the reactions. Blood pressure rose a little with the earlier injections to fall somewhat below normal after a considerable amount of fluid had been injected.

Discussion. Our evidence does not agree with Krogh's observations that a resting muscle contains relatively few open capillaries. We found a relatively large number of capillaries open. The criticism may be offered that mechanical stimulation of the muscle incident to its preparation or the strong illumination caused many capillaries to open. Care was taken to avoid stretching the muscle. Moreover if mechanical stimulation were such a factor, some preparations should be influenced more than others because of the varied amount of manipulation required in different preparations. This did not seem to be the case. Moreover, after the lapse of time stimulated capillaries should subside unless again stimulated. This did not occur.

Illumination did not appear to be much of a factor in keeping capillaries open. We did not use very strong illumination in much of the work; sometimes a 400 watt lamp, occasionally only a 100 watt lamp. Moreover, the rays were not well concentrated and the lamp was placed a few feet distant. The illumination was further reduced by a diaphragm. Even when the observations were begun with the weakest light which made them possible, a large number of capillaries seemed to be open.

Heat was eliminated by a water chamber or by an electric fan.

All capillaries were not open, or at least were not visible, because new

ones came into view at times, as a result of stimulation. It is possible that the pre-capillary vessel was sufficiently constricted to prevent the entrance of corpuscles. It is practically impossible to identify capillaries by their walls alone unless first located by contained corpuscles. But once a capillary is located it can be observed although swept free of its corpuscles.

Capillaries which are open respond to increased pressure passively. Increase in volume of the blood by infusion caused dilatation of the capillaries. This was transient if the volume injected was small.

Independent contractility of capillaries has been demonstrated especially by Krogh in the frog. We have tried to induce constriction of capillaries in the cat's sartorius in a great variety of ways, but with little or no success. Nerve stimulation failed in most instances. All chemical stimuli, almost invariably, caused dilatation except in a few instances of local application. In the case of the latter, the concentration of the substance was much greater than could be tolerated in the general circulation. Moreover, the capillaries often appeared injured.

Epinephrin deserves special mention because such an extensive series of experiments was tried. Constriction of capillaries could be obtained with this substance by local application of a concentration which could not be tolerated systemically if injected in sufficient amount to deliver such concentration to the sartorius. Some of the higher concentrations of intravenously introduced epinephrin caused the corpuscles to disappear from the capillaries. But this was due to pre-capillary constriction, the capillaries actually being dilated much above normal size.

The compensation which occurs in small hemorrhages does not seem to be due to capillary constriction, at least in muscle, for dilatation occurred in all degrees of hemorrhage. Constriction of the spleen and possibly liver may account in part for such compensation. The larger vessels of muscle may constrict.

Reflex stimulation caused by cold produced capillary dilatation.

Thus it is seen that the typical response of the capillaries in skeletal muscle to stimuli is dilatation.

We admit that muscle capillaries must disappear, at times, spontaneously because capillaries not visible before will appear under appropriate stimulation. However, we have not seen capillaries spontaneously disappear.

Capillaries do change in caliber without obliteration which from a physiological viewpoint is important. Stimulation of various sorts causes dilatation. Freedom from stimulation is accompanied by recovery of the original caliber. Thus variations in the blood flow are induced by stimulation. Pre-capillary vessels play a part in variation of flow through the capillaries even going so far at times as to prevent the entrance of corpuscles.

Our observations are limited to skeletal muscle as typified by the sar-

torius. Assuming that all muscle capillaries act in the same way we are dealing with a large portion of the circulation of the body, far larger than that included in the skin or membranes which have been so much studied in the mammal.

Lewis has found that as a rule most if not all of the capillaries in the human skin are open. They are not opening and closing continually.

SUMMARY

The following results have been obtained in the sartorius muscle of the living cat. Observations were made with direct illumination under the high power of the microscope.

1. Faradic stimulation of the femoral nerve usually caused dilatation of the capillaries and sometimes constriction of the arteries and veins.

2. Similar stimulation of the sympathetic nerve also caused dilatation of the capillaries, rarely constriction.

3. Reflexes induced through abdominal skin by cold, heat or electrical stimulation caused dilatation of the capillaries of the sartorius.

4. Mechanical stimulation of the pylorus, to produce shock, caused dilatation of the capillaries of the sartorius.

5. The following substances caused dilatation of the capillaries: Acetic and hydrochloric acids, acetyl choline, amyl nitrite, atropine sulphate, calcium chloride, cocaine, ephedrine, epinephrin, ergotamine, ether, histamine, (ammonium, potassium and sodium) hydroxides, liver extract, nicotine, picrotoxin, pituitrin and urethane.

6. Hemorrhage even in small degree and completely compensated caused dilatation of the capillaries.

7. Infusions caused dilatation.

8. Constriction of the capillaries was rare. Direct application of acetic acid, ferric chloride and ethyl alcohol caused constriction, the first two producing injury to the capillaries. Direct application of 1:10,000 epinephrin caused constriction or disappearance of the capillaries. Local application of ammonium hydroxide caused constriction of the capillaries in one cat.

9. The typical reaction of capillaries to any but harmful stimuli seems to be dilatation.

10. Capillaries have not been observed to open and close.

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OBSERVATIONS ON THE NATURE OF GLOMERULAR ACTIVITY

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The first determinations by a satisfactory method of glomerular capillary pressures were those reported by Hayman (1927) on the frog. He found that in 141 of 181 estimations the pressure lay between 10 and 27 cm. of water; only 11 of 181 measurements gave readings below 10 cm. of water, the average colloidal osmotic pressure of frog's plasma found by White (1924). These results obviously support the view that a filtration process can take place in the glomeruli. The later finding (White, 1928) of a significantly high intracapsular pressure in necturus raised the question as to the amount by which glomerular capillary pressure must exceed plasma colloidal osmotic pressure in order to permit filtration. In order to solve the equation E.F.H. = G.C.P. − I.C.P. − C.O.P., where E.F.H. is the effective filtering head of pressure, G.C.P. the glomerular capillary pressure, I.C.P. the intracapsular pressure and C.O.P. the colloidal osmotic pressure of the plasma, it is desirable to obtain all the data on the same animal. In fact it may be said that the determination of these factors on one and the same animal is essential to a proof or disproof of the possibility of glomerular filtration, which in the past has been rendered highly probable but not directly proved.

Simultaneous, or as nearly so as is possible, determinations on the same animal of the various factors concerned are also of interest for a further reason. The findings by Wearn and Richards (1925) of a higher chloride content of the capsular fluid than of the plasma in the frog and by White (1928) of a higher molecular concentration of the capsular fluid than of the serum in necturus must at present be interpreted as meaning that the glomerular membrane carries out an active secretory process in addition to whatever filtration process may be operating. This being the case, it is conceivable that the glomerular membrane could continue to eliminate fluid by secretion at a time when the E.F.H. had fallen to or below zero, i.e., when the difference between G.C.P. and I.C.P. was exceeded by C.O.P.

METHODS. The principle employed by Hayman for the determination of the glomerular capillary pressure in the frog was used. The nephros-

tome passing from the ciliated neck to the body cavity in necturus introduced a complication. In Hayman's work it was necessary merely to occlude the tubule in order to raise intracapsular pressure to the height determined by the pressure applied through the pipette. In necturus, however, as has been pointed out in a recent paper (White, 1929a) fluid entering the capsule-tubular system even with the tubule occluded will leak out through the nephrostome when the pressure in the system exceeds that which the nephrostome cilia are able to withstand. It is thus apparent that if the leveling bulb attached to the pipette is raised say 20 cm. above the tip of the pipette in the capsule, with the proximal tubule occluded by pressure with a fine glass rod, the resulting intracapsular pressure will be somewhat less than the head of pressure indicated by the height of the leveling bulb. It is further apparent that the extent to which the intracapsular pressure falls below the pressure indicated by the height of the leveling bulb will depend upon the relative resistances of the pipette and of the nephrostome plus that part of the ciliated neck between the capsule and the origin of the nephrostome. If the pipette resistance is large as compared with that through the leak, the intracapsular pressure will be significantly less than the pressure indicated by the height of the leveling bulb. On the other hand, by making the pipette with a relatively wide tip and a short taper its resistance becomes such a small fraction of the total that the intracapsular pressure is only slightly less than the pressure indicated by the height of the bulb.

To determine the amount by which the intracapsular pressure falls below the pressure indicated by the height of the leveling bulb when a pipette of proper dimensions is used the following experiment was carried out. A low-resistance pipette, i.e., one with a short taper and with outside and inside diameters at the tip of 35 and 24μ, respectively, was connected by rubber tubing to a leveling bulb and the system filled with water except for Ringer's solution in the tip end of the pipette. Another pipette, also connected by rubber tubing to a leveling bulb, had a small amount of dye solution sucked into its tip. Both pipettes were inserted simultaneously into a glomerular capsule while both leveling bulbs were at the level of the pipette tips. Capsular fluid was seen to enter the dye-containing pipette, forcing back the dye solution; equilibrium was attained when the leveling bulb of the dye pipette was 3.4 cm. above the tip, i.e., the intracapsular pressure due to the passage of glomerular fluid into the capsule was 3.4 cm. of water. The leveling bulb of the dye pipette (bulb A) was next raised so that enough dye was run in to identify the proximal tubule belonging to this capsule. It was then lowered and the bulb attached to the low resistance pipette (bulb B) was raised to wash the dye out of the capsule and tubule; the proximal tubule was then occluded by pressure with a fine glass rod. Bulb B was then raised 5 cm. above the

tip. The intracapsular pressure, determined by the height of bulb A at which equilibrium between dye solution and capsular fluid was attained, was found to be 5 cm. of water. When bulb B was raised 10 cm. the intracapsular pressure was 10 cm., at 15 cm. the intracapsular pressure was 14.8 to 15 cm., at 20 cm. intracapsular pressure was 19.5 to 19.8 cm., and when bulb B was at 25 cm. the intracapsular pressure was 24.3 to 24.7 cm. The exact correspondence between intracapsular pressure and height of bulb B up to 10 cm. is due to the fact that the nephrostome cilia can withstand this great a pressure, i.e., there is no leak through the nephrostome. When bulb B is at heights of 15 cm. or greater there is a slight leak through the nephrostome. This leak is so slight, however, as compared with the volume of fluid which is capable of passing the pipette resistance at the corresponding pressures that the intracapsular pressure is lowered only insignificantly. These results could be predicted from a rough calculation of the relative resistances of the pipette and of the route of the leak, the former being certainly nor more than 5 per cent of the latter. It may be pointed out here that if the technique for occluding the nephrostome, described later in the paper, had been perfected at this stage, this determination of the error introduced by the nephrostome leak would not have been necessary.

Having established that the height of the leveling bulb attached to a pipette of appropriate dimensions can be taken as a reliable measure of intracapsular pressure it was a simple matter to determine the applied pressure necessary to stop or to affect the glomerular capillary blood flow. In the experiments reported in the protocols a single pipette was used to determine the preëxisting intracapsular pressure and the applied pressure necessary to stop or to affect the glomerular flow. The pipette, containing in its tip a small volume of half-saturated trypan blue solution, was inserted into a capsule and the intracapsular pressure determined in the usual way. The bulb was now raised a few centimeters and the dye solution run down the tubule to identify it. The bulb was kept raised until the capsule was washed clear of the dye, this being necesary in order that the subsequent observations of the glomerular tuft could be made satisfactorily. It was found that on using saturated trypan blue solution the capsule was frequently stained so that even prolonged washing out failed to clear it sufficiently to permit satisfactory observations of the changes in glomerular flow which were taken as the criterion of glomerular capillary pressure. This difficulty was greatly reduced by the use of half-saturated and later of about fifth-saturated trypan blue solution, which is still sufficiently colored to permit easy visualization. After the dye had been washed out the proximal tubule was occluded by pressure with a fine glass rod and the leveling bulb raised until the changes in glomerular flow were seen. The interpretation of these changes will be discussed after the data have been presented.

The method of determining the third factor of the equation, the colloidal osmotic pressure, may next be considered. Collodion sacs prepared according to the technique of Krogh and Nakazawa (1927) were first used. Sacs prepared by Krogh's B-4 technique had minute numbers ranging from 150 to several thousand; on the average the minute numbers were higher than those reported by Krogh and Nakazawa, indicating less permeable membranes than they obtained. A high degree of uniformity, as indicated by correspondence of the minute numbers, is not, however, necessary. Thus, a sac with the minute number of 150 gave the same colloidal osmotic pressure, 29 cm. of water, on normal dog plasma as did one with the minute number of 800. When the same plasma was put into a sac dried somewhat longer, with the unusually high minute number of 23,000, the meniscus still rose against a counter pressure of 60 cm. of water, the limit of the manometer. The outside fluid (Ringer's solution) is free from protein when tested by Spiegler's reagent and by the molybdic acid reagent used in Brigg's phosphate determination (1922), which was found to be a much more sensitive test for protein than is Spiegler's reagent. The protein-free state of the outside fluid obtains when sacs with minute numbers of 150 or higher are used; none of my sacs had minute numbers less than 150. It thus appears that a sac with a minute number of 150 will not let pass any plasma constituent to which a sac with a minute number of 800 is impermeable, although a much tighter membrane with a minute number of 23,000 retains additional osmotically active substances. In other words, a membrane which is just tight enough to hold back all the protein can be made considerably tighter without any resultant increase in osmotic pressure.

While Krogh's method was thus found to be practicable it seemed more convenient to use a ready-made membrane such as cellophane. Verney (1926, 1928) has described an apparatus built upon the principles employed by Govaerts (1924) in which a cellophane membrane is used. Two osmometers, each with a capacity of about 0.3 cc., were made and gold plated. Cellophane plain 600, with a thickness of 0.004 to 0.005 cm., was used. Since Krogh and Nakazawa showed that considerable variations in temperature do not significantly affect the colloidal osmotic pressure, the aseptic precautions employed by Verney were dispensed with, the osmometers being set up in a cold room at a temperature of 6° to 8°C. The osmometers with their washers were boiled occasionally but no attempt was made to carry out strict aseptic technique in collecting or handling the blood. No antiseptic was added. That no significant bacterial growth took place under these conditions is shown by the facts that the serum remained clear and that the figures obtained on normal sera correspond with those obtained by Verney. Additional evidence is given by the following experiment. The nonprotein nitrogen of a fresh sample of dog

serum was found to be 33 mgm. per 100 cc. The osmometers were filled with this serum, both inside and outside chambers containing serum to prevent dilution by diffusion, and left in the cold room for 24 hours. The nonprotein nitrogen of the contents of one osmometer after this stay in the cold room was determined as 34 mgm. per 100 cc., of the other as 34.8 mgm. per 100 cc., showing that no breaking down of proteins had taken place. The Ringer's solution outside the membrane in the colloidal osmotic pressure measurements was invariably protein-free. The cellophane used has a minute number of 300 to 500, on the average. Its convenience and uniformity make it, to my mind, the membrane of choice.

TABLE 1

SERUM	COLLOIDAL OSMOTIC PRESSURE	TOTAL NITROGEN	NON-PROTEIN NITROGEN	PER CENT PROTEIN	COLLOIDAL OSMOTIC PRESSURE IN CM. H_2O FOR EACH PER CENT PROTEIN
	cm. H_2O	*mgm. per 100 cc.*	*mgm. per 100 cc.*		
Normal dog*	33				
Normal rat	26, 26.5	870	28	5.26	5.0
Normal rat	20.5, 21	870	25	5.28	3.9
Normal dog	27, 28	1,110	35	6.72	4.1
B. Cancer of bladder with uremia	33, 34	1,250	164	6.78	4.9
H. Glomerulonephritis	30.5, 29	1,165	37	7.06	4.2
S. Edema, nutritional?	13, 14	450	22	2.68	5.0
S. Edema, nutritional?	19, 19.5	650	28	3.89	5.0
S. Edema, nutritional?	14.7	495	33	2.89	5.1
S. Edema, nutritional?	19	492	20	2.95	6.4

* This sample is plasma, 0.2 per cent $(COOK)_2 \cdot H_2O$ being added to the blood. All the others are sera. Pairs of figures in the colloidal osmotic pressure column indicate duplicate determinations.

In table 1 are given the colloidal osmotic pressure and nitrogen figures of various mammalian sera, using cellophane membranes.

The necturi used in the experiments reported in the protocols were prepared sometimes by pithing the brain without an anesthetic, sometimes by immersion for a few minutes in 2.5 per cent urethane solution, the head and gills being immersed in 0.3 per cent urethane during the experiment.

Protocols. February 2. Male. Circulation very poor. Few glomeruli showed slow circulation with capillaries distended, others no circulation. Pipette inserted into capsule containing only a little fluid, intracapsular pressure not measurable. Flow stopped at pressure of 6.5 cm., continuous at 6 cm. Colloidal osmotic pressure of serum 5.0 cm. of water, total N 213 mgm. per 100 cc., nonprotein nitrogen (NPN) 15 mgm., giving 1.24 per cent protein and 4.0 cm. of water pressure for each per cent protein.

February 5. Female. Circulation poor, a few glomeruli showed fair circulation. Intracapsular pressure 3 mm. of water, flow stopped at 8 cm., continuous at 7.2 cm. Colloidal osmotic pressure 5.5 cm. Total N 233 mgm., NPN 13, giving 1.38 per cent protein.

February 8. Male. Circulation excellent. Practically all capsules well filled, capillaries narrow, flow rapid. First capsule entered showed intracapsular pressure of 2.8 cm., flow stopped at 24 cm. At 23.5 cm. cells entered tuft and moved on systole only. When pressure was lowered to 20 cm. continuous flow was established, although slowed on diastole, all loops but one opening simultaneously. After about 40 seconds, with pressure maintained at 20 cm., this loop also established continuous flow. Pressure again raised to 24 cm., cells entered on systole and in a few seconds flow was continuous through all loops at 24 cm.; in about 30 seconds all flow stopped again, tuft completely free of cells. As intracapillary pressure fell cells in one loop appeared to be squeezed toward the afferent vessel, in the other loops toward the efferent vessel. Cell-free tuft still pulsates at each systole. Individual loops of tuft shrink down toward arterial pole, the loops seeming to be folded longitudinally, not merely compressed *in situ*. This would indicate that the systolic pulsations of the tuft which continue when the intracapsular pressure is high enough to squeeze out all the cells are due merely to the systolic impact of the pulse in the afferent vessel rather than being due to the systolic entrance of cell-free plasma. The cell-free tuft is seen as a yellowish-white irregularly outlined mass occupying a much smaller fraction of the capsule volume than when circulation is active. The pressure was continued at 24 cm. and after 2 or 3 minutes systolic entrance and movement of cells was seen, lasting about 30 seconds; the cells were then again squeezed out. The tuft was observed about 5 minutes more with pressure maintained at 24 cm. but no further entrance of cells occurred. Pressure lowered to 22 cm., systolic movement reappeared; continuous circulation (through all loops simultaneously) although slowed on diastole when pressure was lowered to 19 cm. Pipette inserted into another capsule only moderately filled. Capillaries here were more dilated, flow slower but all loops showed circulation. Intracapsular pressure 0.5 cm. Flow stopped at 11 cm., continuous at 9.8 cm. Colloidal osmotic pressure of serum 8.5 cm. Total N 287 mgm., NPN not determined, assume it 15 mgm., giving 1.70 per cent protein.

February 11. Male. Circulation only fair, a few glomeruli show no circulation and none show the brisk circulation with cells moving rapidly through narrow capillaries, which is considered normal. Most glomeruli show cells moving more or less rapidly, some through all loops, some through part. Most capillaries are widened and no capsules are fully distended, although there is some fluid-filled space in all where circulation is even moderate. First capsule entered had intracapsular pressure of 0.3 cm., flow stopped at 10.5 cm., systolic movement at 10.3 cm., continuous at 9.7. Second capsule, intracapsular pressure not measurable, flow stopped at 7 cm., continuous at 6.5. Serum colloidal osmotic pressure 7.5 cm. Total N 327, NPN 16 mgm., giving 1.95 per cent protein and 3.9 cm. for each per cent protein.

February 13. Circulation poor, best capsule entered had intracapsular pressure not measurable. Flow stopped at 9 cm., continuous at 8.5. Second capsule, circulation quite feeble. Dye injected into capsule did not wash out, i.e., there was apparently no fluid entering capsule from glomerulus. Flow stopped at 3 cm., continuous at 2.7. Colloidal osmotic pressure 7.2 cm. Total N 292, NPN 18 mgm., giving 1.71 per cent protein and 4.2 cm. for each per cent protein.

February 14. Male. Circulation fair. Took one rather active glomerulus, intracapsular pressure 1 cm. Flow stopped at 12 cm., continuous at 11.2 cm. Second capsule, circulation slow, intracapsular pressure hardly measurable, perhaps 0.1

to 0.2 cm. Flow stopped at 7, continuous at 6.5 cm. Aortic pressure 17.5 cm. Colloidal osmotic pressure 6.5 cm. Total N 278, NPN 12 mgm. giving 1.66 per cent protein.

February 23. Male. Circulation good, intracapsular pressure 2 cm. Flow stopped at 19 cm., continuous at 17. Aortic pressure 30 cm. Colloidal osmotic pressure 7.8 cm. Total N 310, NPN 10 mgm., giving 1.88 per cent protein.

February 26. Female. Circulation fairly good. Intracapsular pressure 1.4 cm., flow stopped at 18, continuous at 15.5 cm. Pipette put into either the afferent vessel or the stem from which it springs, pressure 18 to 19 cm. Another capsule had intracapsular pressure of 0.8 cm. Flow stopped at 17, continuous at 14 4. A third with feeble circulation had intracapsular pressure perhaps 0.1 to 0.2 cm., flow stopped at 8, continuous at 7.4 cm. Colloidal osmotic pressure 14.2 cm. Total N 586, NPN 12 mgm., giving 3.5 per cent protein.

February 28. Male. Circulation excellent. First capsule, intracapsular pressure 2.4 cm., flow stopped at 28, continuous at 25 cm. Second capsule, circulation less rapid, intracapsular pressure 0.8 cm., flow stopped at 14.5, continuous at 13 cm. Third capsule, flow sluggish, capillaries dilated. Intracapsular pressure not measurable, flow stopped at 8.5, continuous at 8 cm. Colloidal osmotic pressure 11 cm. Total N, 454, NPN 14 mgm. per 100 cc., giving 2.75 per cent protein.

March 2. Female. Circulation excellent in a few capsules, fair in some, poor in most. First capsule, best circulation, intracapsular pressure 2.0 cm., flow stopped at 22, continuous at 20 cm. Second capsule, circulation fair, intracapsular pressure 0.8 cm., flow stopped at 15, continuous at 14 cm. Third capsule, circulation poor, intracapsular pressure not measurable. Flow stopped at 7, continuous at 6.5 cm. Aortic pressure 33 cm. Colloidal osmotic pressure 15.8 cm. Total N 588, NPN 16, giving 3.5 per cent protein.

March 5. Female. Circulation fair in most capsules, poor in others. In one of the most active capsules, intracapsular pressure 1.2 cm., flow stopped at 16, continuous at 14.8 cm. Another showed intracapsular pressure 1 cm., flow stopped at 15, continuous at 14 cm. Third capsule, circulation poor, intracapsular pressure not measurable, flow stopped at 7.8, continuous at 7.3 cm. Aortic pressure 25 cm. Colloidal osmotic pressure 10.8 cm. Total N 415, NPN 15 mgm., giving 2.5 per cent protein.

In all of these experiments a small volume of dye solution was injected into the capsule just after determining the intracapsular pressure and its fate observed. In every case reported in the protocols, except the second capsule on February 13th, the dye was washed out of the capsule into the tubule and replaced by clear fluid. The activity of the cilia in the ciliated neck of the tubule excludes the possibility that fluid can enter the capsule from the tubule or from the body cavity via the nephrostome (White, 1929a). In other words, the observations prove that fluid is being eliminated into the capsule by the glomerulus. The rate at which the injected dye left the capsule and was replaced by clear fluid bore a definite relation to the activity of glomerular circulation, varying from perhaps 30 seconds with the most active glomeruli to several minutes with the sluggish ones.

The aortic pressure was determined in some of the experiments by

inserting into the aorta near the heart a long glass tube of 500μ inside diameter, drawn to a tip of about 150μ outside diameter and bent at right angles near the tip. The tube before insertion was filled with Ringer's solution to a length of about 40 cm. After the tube had entered the aorta, the column fell until its height corresponded to the aortic pressure plus the capillarity of the tube. The latter was determined and subtracted from the total height, giving the pressure in the unobstructed aorta. It will be noted that this pressure reading was taken proximal to the gills; the pressure in the abdominal aorta is undoubtedly lower.

The lowest pressure which just stops all flow through the glomerulus is interpreted as being just above the lateral pressure during systole in the arteriole from which the afferent vessel springs; the slightest lowering of this pressure will, of course, permit systolic entrance of cells, which has been taken as a reasonably accurate index of systolic pressure in the afferent vessel. Hayman's statement, "The minimum intracapsular pressure at which no cells enter the tuft is appreciably higher than the maximum intracapsular pressure at which entrance of cells is permitted," apparently refers to a decreasing pressure in the first instance and an increasing pressure in the second; I have made no observations on this point. Hayman has regarded the highest pressure which just permits continuous flow through at least one capillary loop as a measure of diastolic capillary pressure. This criterion seems to me, however, to be more nearly a measure of the diastolic pressure at the origin of the afferent vessel from its stem; the diastolic pressure in a branched vessel, all of whose branches are in parallel and quickly reunite to form a common vessel as with the glomerulus, will rise on obstruction to the diastolic pressure of the afferent vessel, just as with an unbranched vessel. When flow is established there is, of course, a pressure drop from afferent vessel to capillary, so that the above criterion gives a figure somewhat lower than diastolic afferent vessel pressure but, according to my interpretation, not necessarily the same as the diastolic glomerular capillary pressure. On this interpretation what Hayman regards as the pressure drop from afferent vessel to glomerular capillary is rather a rough measure of and somewhat greater than the pulse pressure in the afferent vessel. If, then, we refuse to accept the highest pressure which permits continuous glomerular flow as a criterion of diastolic capillary pressure and consider it as giving a figure somewhat, and to an unknown extent, below diastolic afferent vessel pressure, we must consider the probable error introduced in adopting it as a measure of mean glomerular capillary pressure.

The only direct observations with which I am acquainted on the pressure gradient from unobstructed arterioles to venules are those by Landis (1926, 1928) who found that in the frog's mesentery the pressure in the arteriolar end of the capillary (to which the glomerular capillaries are

analogous) is only slightly below the diastolic pressure in the arteriole. Since the efferent vessel from the glomerulus is smaller than the afferent it is highly probable that the difference between diastolic afferent vessel pressure and mean glomerular capillary pressure is even less than in the mesenteric arteriolar-capillary transition. It thus appears to me quite permissible to accept the criterion adopted, which is the only end-point that can be used, as a reasonably accurate measure of mean glomerular capillary pressure, recognizing that it gives maximal figures.

I cannot agree with Hill and McQueen (1921, 1928) that the "momentary check index" gives sufficiently high figures for arteriolar or capillary pressures or that the highest pressure permitting continuous glomerular capillary flow is considerably above mean glomerular capillary pressure and represents rather a banked-up arterial pressure. In regard to the first point, it seems to me that of necessity the first venous obstruction will produce a transient effect on the flow in the capillaries and arterioles. As to the second point, the method which Hayman and I have used may, it is granted, give figures somewhat higher than the actual mean glomerular capillary pressure; reasons for believing that the error is not great have been given. Another point upon which Hill has long insisted, that the intracapsular pressure must be only slightly inferior to the intracapillary pressure, is definitely refuted by the measurements reported in this and in previous papers (White, 1928, 1929b).

The data of the protocols show that in glomeruli with active circulation the E.F.H. as calculated from the equation given at the beginning of this paper is sufficient to permit a filtration process. If we take the pressure which just permits continuous flow as a measure of the mean glomerular capillary pressure we see, for example, that in the first capsule on February 8th the E.F.H. = (20 − 2.8) − 8.5 = 8.7 cm., on February 23rd E.F.H. = (17 − 2) − 7.8 = 7.2 cm., in first capsule on February 28th E.F.H = (25 − 2.4) − 11 = 11.6 cm., etc. With the less active glomeruli the E.F.H. is lower, as with the second on February 8th, E.F.H. = (9.8 − 0.6) −8.5 = 0.7 cm., with the second on February 28th E.F.H. = (13 − 0.8) − 11 = 1.2 cm., etc. With still less active glomeruli the E.F.H. may be zero or below; with the second capsule on February 11th E.F.H. = (6.5 − 0) − 7.5 = −1 cm., second on February 14th E.F.H. = (6.5 − 0.1) −6.5 = −0.1 (or 0), third on February 26th E.F.H. = (7.4 − 0.1) − 14.2= −6.9 cm., etc. Nevertheless, in this last group fluid is being eliminated by the glomeruli into the capsules, as is indicated by the injected dye leaving the capsule and being replaced by clear fluid. These facts demonstrate that filtration is taking place through a normally actively circulating glomerulus and indicate further that when the circulation is depressed to the extent that filtration is no longer possible through a given glomerulus, the glomerular membrane can continue to pass fluid through the capsule

by a process other than filtration, presumably active secretion. The demonstration of filtration through a glomerulus with normally active circulation does not, of course, preclude a simultaneous secretory process; the findings of Wearn and Richards on the chlorides and of White on the molecular concentration of the capsular fluid make such a secretory process in active glomeruli highly probable. The other point indicated by the present data, that the secretory process may continue when filtration is no longer possible, may be objected to on the following grounds. It might be supposed that the membrane of a glomerulus whose circulation was depressed to the extent that filtration was no longer possible, according to the E.F.H. as calculated by the above equation, but which continued to eliminate fluid into the capsule, had become permeable to protein. If this were the case the effective C.O.P. of the serum would be reduced to the difference of the colloidal osmotic pressures of the serum and of the capsular fluid, and if this reduction of effective C.O.P. were of sufficient magnitude it is conceivable that the E.F.H. could still be positive, even in the third group referred to above.

An additional series of experiments was therefore carried out to determine whether or not a glomerulus with active circulation, and therefore with capillary walls of normal permeability, can continue to eliminate fluid when the E.F.H. is zero or below. In this series a negative E.F.H. was attained by raising artificially the intracapsular pressure rather than by selecting glomeruli with a low glomerular capillary pressure. This was accomplished as follows. The pipette connected to a leveling bulb and containing an air-bubble indicator was inserted into a capsule which was then closed by occluding the proximal tubule and the nephrostome. The intracapsular pressure can then be raised to any desired height by raising the leveling bulb. After the glomerular capillary pressure has been determined the intracapsular pressure is set at various levels below the glomerular capillary pressure and the elimination of fluid by the glomerulus observed by measuring the progress of the air-bubble indicator in the pipette.

Numerous schemes for rendering the capsule a closed system were tried without success. First, droplets of mercury were injected into the capsule in the attempt to have them lodge in the ciliated neck and occlude it. Next, a 1.5 per cent agar jell colored with trypan blue was melted and dropped on the ventral surface of the kidney with the idea that it would be swept in by the nephrostomes and solidified in them and in the ciliated necks beyond the nephrostomes. When the excess agar was washed off the colored jell could be seen in the nephrostomes and tubules but when Ringer's solution was dropped on the agar was washed down; apparently it did not form a solid plug. The same scheme was tried with melted cocoa butter stained with Sudan IV, but this would not enter the nephrostomes.

The method finally adopted was to occlude the nephrostome by searing it with a loop of no. 30 nichrome wire heated by an electric current. The proximal tubule was closed sometimes in the same way, sometimes by pressure with a fine glass rod. If the wire is not heated too hot (the current was supplied by a lead storage battery and regulated by a rheostat) and the point of searing is not too close to the glomerulus the circulation in the glomerulus is not affected. Closing the capsule could be accomplished most directly by searing shut the ciliated neck between capsule and origin of nephrostome. When this was done, however, there usually resulted a marked impairment of circulation of the glomerulus in question, so this plan was abandoned in favor of the double sealing at points farther from the glomerulus.

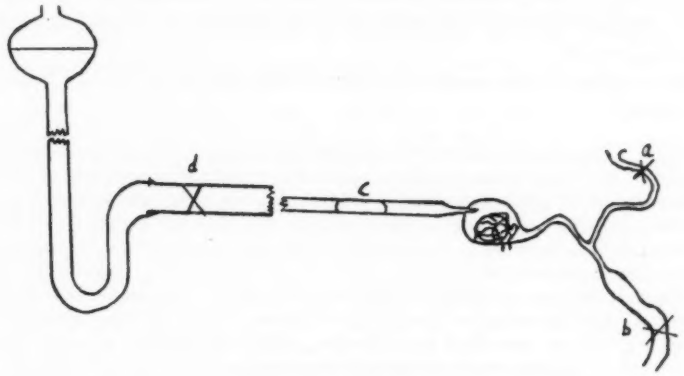

Fig. 1

The procedure finally adopted was as follows. A little dye solution (one-fifth saturated trypan blue) is drawn into the tip of a pipette and injected into the capsule, the proximal tubule, after being identified by the injected dye, is occluded by a fine glass rod and the leveling bulb raised until dye is forced out of the nephrostome which is then closed by searing. The glass rod is then removed, the pipette removed from the capsule and the dye allowed to wash out of capsule and tubule; the proximal tubule is then seared shut or may be closed again by the glass rod during the period of raised intracapsular pressure. Another pipette has been drawn with a uniform inside diameter near the tip of 300 to 360μ. This is connected with a leveling bulb supported on a Zimmermann stativ, a stopcock being interposed. The leveling bulb, connecting system and pipette out to about 1 cm. from the tip are filled with water. Ringer's solution is now drawn up into the tip of the pipette, leaving an air-bubble between Ringer's and water, and the pipette inserted into the closed capsule. The glomeru-

lar capillary pressure is next determined, sufficient Ringer's having been drawn into the pipette to distend the capsule. Intracapsular pressure is next set at any desired height, determined by the height of the leveling bulb. The rate of elimination of glomerular fluid at each intracapsular pressure is determined by measuring the rate of progress of the meniscus between air-bubble and Ringer's solution, a micrometer ocular with each space corresponding to 38.4μ being employed. At the end of the experiment the inside diameter of the pipette shaft is measured and the volume of fluid eliminated per unit time calculated. In figure 1, *a* and *b* are the points of occlusion of nephrostome and proximal tubule, respectively; *c* is the air-bubble indicator and *d* is a stopcock. The drawing is not to scale. It was established that when the diameters of the pipette shaft at the two ends of the air-bubble are the same the air-bubble moves in response to the slightest pressure difference with the pipette tip free in a horizontal column of water.

The results of this series of experiments are given in the following protocols.

April 8. Female. Glomerular circulation stopped at 12 cm., just continuous at 11.2 cm. H_2O. When intracapsular pressure was 8 cm. the bubble was practically stationary, moved down very slowly. When I.C.P. was 6 cm. bubble moved up two spaces in 5 minutes. (Inside shaft diameter 400μ, therefore $0.2 \times 0.2 \times \pi \times 0.038 \times 2 = 0.01$ cu. mm. in 5 minutes.) Bulb next lowered to level of tip, fluid rose 26 spaces or 0.13 cu. mm. in 10 minutes. C.O.P. = 7.5, 7.8, average 7.7 cm. Total N 315, NPN 15, protein N 300 mgm. per 100 cc. or 1.88 per cent protein. When bulb is 6 cm. above pipette tip, E.F.H. $= 11.2 - 6 - 7.7 = -2.5$ cm. and at this time glomerulus is eliminating 0.01 cu. mm. fluid in 5 minutes. With bulb at level of tip E.F.H. $= 11.2 - 0 - 7.7 = 3.5$ cm. and glomerulus is eliminating 0.13 cu. mm. in 10 minutes.

April 11. Female. Circulation unusually strong. Glomerular circulation stopped at 30 cm., just continuous at 27 cm. Bubble moved down very slowly when I.C.P. was 25 cm., moved up 4 spaces in 5 minutes when I.C.P. was 20 cm. (Inside shaft diameter = 210μ, therefore $0.1 \times 0.1 \times \pi \times 0.038 \times 4 = 0.005$ cu. mm. glomerular fluid in 5 minutes.) With bulb 10 cm. above pipette tip fluid rose 47 spaces or 0.057 cu. mm. in 5 minutes. Bulb next lowered to level of pipette, fluid rose 90 spaces or 0.11 cu. mm. in 3 minutes. G.C.P. redetermined at end of experiment, flow stopped at 26 cm., just continuous at 24. C.O.P. = 13.8, 14.6, average 14.2 cm. Total N 559, NPN 20, protein N 539 mgm. per 100 cc. or 3.38 per cent protein. Elimination of glomerular fluid began when I.C.P. was 20 cm. or E.F.H. $= 27 - 20 - 14.2 = -7.2$ cm. H_2O.

April 17. Male. Glomerular flow stopped at 17 cm., continuous at 15.5. When bulb was 13 cm. above the tip, bubble moved down at barely perceptible rate, less than 1 space in 5 minutes. With bulb at 10 cm. bubble moved up 1 space in 5 minutes or 0.003 cu. mm., since inside shaft diameter was 300 μ. With bulb at 5 cm., i.e. I.C.P. 5 cm., fluid moved up pipette 4 spaces or 0.012 cu. mm. in 5 minutes. With bulb at 2.5 cm. fluid moved up 20 spaces or 0.06 cu. mm. in 5 minutes. Definite oscillations of bubble seen here, shifting back at times almost a whole space but general trend upward. G.C.P. redetermined at end of experiment, flow stopped at 16.5 cm., continuous at 15.5. C.O.P. 10, 9.4, average 9.7 cm. Total N 390, NPN

not determined, call it 20, protein N 370 mgm. per 100 cc. or 2.31 per cent protein. Elimination of glomerular fluid began when I.C.P. was 10 cm. or E.F.H. = 15.5 − 10 − 9.7 = −4.2 cm. H_2O.

April 19. Male. Glomerular flow stopped at 13 cm., continuous at 12. Bubble moved down less than 1 space in 5 minutes when I.C.P. was 10 cm., stationary at 8.5 to 9 cm. When I.C.P. was 7 cm. bubble moved up 1 space in 5 minutes or 0.003 cu. mm. (pipette shaft inside diameter 320 μ). With bulb at level of tip, i.e., I.C.P. zero, bubble moved up 24 spaces in 5 minutes or 0.075 cu. mm. G.C.P. redetermined at end, flow stopped at 12 cm., continuous at 11. C.O.P. 9.0, 8.4, average 8.7 cm. Total N 320, NPN not determined, call it 20, protein N 300 mgm. per 100 cc. or 1.88 per cent protein. Elimination of glomerular fluid began when I.C.P. was 7 cm. or E.F.H. = 11.5 − 7 − 8.7 = −4.2 cm. H_2O.

April 23. Female. Glomerular flow stopped at 14.5 cm., continuous at 13. Bubble moved down very slowly with bulb at 12, 11 and 10 cm., stationary at 9 to 9.5. Moved up very slowly (about 1 space in 5 minutes) with bulb at 8 to 8.5 cm., moved up 2 spaces in 5 minutes, or 0.008 cu. mm. (pipette diameter 360 μ) with bulb at 7 cm. When bulb is lowered to 2 cm. fluid level rose 22 spaces or 0.086 cu. mm. in 5 minutes. Glomerular flow at end of experiment stopped at 14 cm., continuous at 13. C.O.P. = 9.2, 9.8, average 9.5 cm. Total N 346, NPN 15, protein N 331 mgm. per 100 cc. or 2.07 per cent protein. Elimination of glomerular fluid began when I.C.P. was 8 to 8.5 cm. or E.F.H. = 13 − 8.3 − 9.5 = −4.8 cm.

April 25. Male. Glomerular flow stopped at 19 cm., continuous at 17. Bubble moved down very slowly when bulb was at 14 cm., stationary at 13 to 14, moved up very slowly (less than 1 space in 5 minutes) at 12 cm., moved up 2 spaces in 5 minutes or 0.006 cu. mm. (pipette diameter 330 μ) at 10 cm., 3 spaces or 0.009 cu. mm. in 5 minutes at 8 cm. Bulb next lowered to 3 cm., fluid moved up into pipette 29 spaces or 0.083 cu. mm. in 5 minutes. G.C.P. redetermined, flow stopped at 18, continuous at 16. C.O.P. 11.4, 11.8, average 11.6. Total N 440, NPN 13, protein N 427 mgm. per 100 cc. or 2.77 per cent protein. Elimination of glomerular fluid began when I.C.P. was 12 cm. or E.F.H. = 16.5 − 12 − 11.6 = −7.1 cm.

April 26. Male. Glomerular flow stopped at 16, continuous at 14.5 cm. Bubble moved down slowly when I.C.P. was 12 and 11 cm., stationary at 10, moved up slowly (about 1 space in 5 minutes) at 9 cm., 2 spaces in 5 minutes or 0.008 cu. mm. (inside shaft diameter 360μ) at 8 cm. G.C.P. again determined, flow stopped at 15, continuous at 14 cm. When bulb was lowered to 2 cm. bubble moved up 18 spaces or 0.07 cu. mm. in 5 minutes. C.O.P. = 9.2, 10, average 9.6 cm. Total N 374, NPN 14, protein N 360 mgm. per 100 cc. or 2.25 per cent protein. Elimination of glomerular fluid began when I.C.P. was 9 cm. or E.F.H. = 14.3 − 9 − 9.6 = −4.3 cm.

It is clear from the above protocols that the glomerulus can continue to eliminate fluid when the pressure relationships are such that filtration presumably cannot occur. Our interpretation of the results is that the glomerular fluid formed when the E.F.H. is less than zero is formed by a secretory process. The rate of formation of glomerular product when the E.F.H. is less than zero is seen to be quite low; there is apparently a sudden increase when the E.F.H. becomes positive.

The point might be raised that the C.O.P. as determined with the cellophane membrane is higher than that which would be exerted across the glomerular capillary wall and membrane and that the true E.F.H. may

thus be positive when, according to the equation, it is negative. This view would demand that the physiological C.O.P. is considerably lower than that exhibited across a cellophane membrane, i.e., that the glomerular membrane is much more permeable than a cellophane membrane. For the following reasons we do not believe that this is true. First, the glomerular membrane in necturus is tight enough to hold back all the protein; this has been demonstrated by direct observation (White and Schmitt, 1926). Second, the cellophane membrane used also holds back all the protein (in every one of the C.O.P. determinations the outside Ringer's solution was protein-free when tested with Brigg's molybdic acid reagent which gives a cloud with necturus serum diluted 1 to 1400) but according to Verney (1927) is completely permeable to all of the other blood constituents tested. Third, the scanty and rather indirect evidence which does exist as a basis for making any guess as to the relative permeabilities of the membranes would indicate that the glomerular membrane is perhaps less permeable; this evidence is the finding (Schmitt and White, 1928) that only about one-fourth to one-third of the plasma inorganic phosphate can pass the glomerular membrane of necturus, while Grollman (1927) found that 85 per cent of the inorganic phosphate in frog's and 50 to 90 per cent in terrapins' serum would pass the collodion membranes he used. These collodion membranes were presumably of about the same permeability as the cellophane membranes; the evidence for this statement is that figures for the colloidal osmotic pressure of normal mammalian serum obtained with collodion sacs prepared by Grollman's technique agree very well with those obtained with cellophane membranes. Fourth, as was shown in an earlier part of this paper, a membrane can be considerably more than just tight enough to hold back all the proteins and still not give a higher colloidal osmotic pressure of normal serum than will a membrane which is just tight enough. To sum up, there is no reason to believe that the cellophane membrane is any tighter than the glomerular membrane, there is even some slight evidence that it is less tight and finally, even if it were considerably tighter it would not necessarily show a higher colloidal osmotic pressure reading. If, then, we are willing to accept the observed figures as being even a reasonable approximation to the colloidal osmotic pressure exhibited across the glomerular membrane, it follows that the glomerulus, in addition to its filtration activity, can eliminate fluid by a process other than filtration.

The slow movement of fluid out of the pipette when the applied pressure is only slightly below the glomerular capillary pressure may be mentioned in closing. This is definitely not due to a leak, as can be shown by applying pressure with the system filled with dye. Further evidence against a leak is that a pressure can be found at which the bubble remains stationary, a pressure which is still considerably above atmospheric. The slow passage

of fluid from pipette into capsule apparently means an absorption of water under the abnormally high pressure, either by the glomerular capillaries or by that part of the tubule above the occlusion or by both.

SUMMARY

The capillary pressures in 19 glomeruli in 16 necturi in which the circulation was classified by inspection as "fair" to "excellent" ranged from 8.5 to 27 cm. H_2O. The effective filtering head of pressure for these 19 glomeruli was positive, i.e., the glomerular capillary pressure exceeded the sum of intracapsular pressure and colloidal osmotic pressure of serum, the intracapsular pressures in this series ranging from 0.1 or 0.2 to 2.8 cm. H_2O and the serum colloidal osmotic pressure from 6.5 to 14.2 cm. H_2O.

In 9 other glomeruli with sluggish circulation the pressure was less than the sum of the intracapsular pressure and colloidal osmotic pressure, i.e., the filtering head of pressure was less than zero. Nevertheless, these glomeruli were eliminating fluid as shown by the fact that dye injected into the capsules was washed down into the tubules. From these later observations the conclusion might be drawn that the glomerulus can eliminate fluid by a process other than filtration; an objection to this conclusion might be that the walls of those capillaries with sluggish circulation were abnormally permeable, so that the effective colloidal osmotic pressure was less than the observed.

Therefore, in an additional series of experiments negative effective filtering pressure heads were obtained by raising artificially the intracapsular pressure while the glomerular capillary pressure was normal (and therefore the permeability of the glomerular capillary walls and membrane was presumably normal) rather than by selecting glomeruli with sluggish circulation. In 7 capsules with glomerular capillary pressures ranging from 11 to 27 cm. H_2O, fluid entered the pipette from the capsule at a time when the effective filtering head of pressure was less than zero, i.e., when the sum of intracapsular pressure and colloidal osmotic pressure exceeded the glomerular capillary pressure. The findings are interpreted as evidence that the glomerular membrane both filters and secretes.

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EFFECT OF DEFIBRINATED BLOOD ON THE CONTRACTION OF SMOOTH MUSCLE

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In recent years the hypothesis that histamine plays a prominent part in traumatic shock has attracted much attention. The symptoms produced by the injection of a massive dose of histamine into a dog have a marked resemblance to what is seen in traumatic shock, but practically any drug which will induce a prolonged fall in blood pressure may bring out a picture not unlike surgical shock. Many, if not all, of the symptoms of shock are dependent on the low blood pressure. Thus, shock may be secondary to hemorrhage, and the resemblance of shock to hemorrhage may be more than superficial, since the injection of even more blood than was lost in the bleeding may not be efficacious in permanently restoring the animal. Anoxemia may induce a condition very much like traumatic shock in its more obvious manifestations. It is apparent, therefore, that, in the absence of more evidence than is today available in its favor, histamine must not be entertained too seriously in a consideration of the etiology of shock. In support of this contention, I would urge what has already been pointed out by Herrick and Markowitz (1929): that although it is extremely easy to induce shock in a rabbit, this animal nearly always responds by a rise in blood pressure to the injection of histamine. This much can be said in favor of the histamine hypothesis: there is present in the lung, in the liver, in the spleen and in the bone marrow, enough histamine (probably in a free state) to cause considerable injury to an organism were this suddenly liberated into the circulation.

If one is going to look for a humoral (as opposed to reflex) mechanism for shock, there is a clear-cut lead in the well-known fact that shed blood rapidly acquires intense vasoconstrictor properties. In the past it has been assumed that a substance with vasoconstrictor properties will necessarily cause a rise in blood pressure on intravenous injection. However, the fallacy of this reasoning is at once evident when one considers that histamine is intensely vasoconstrictive. It seems, therefore, not an impossible thesis that the vasoconstrictor substance of shed blood may cause

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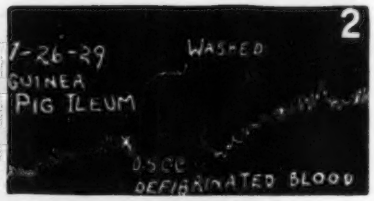
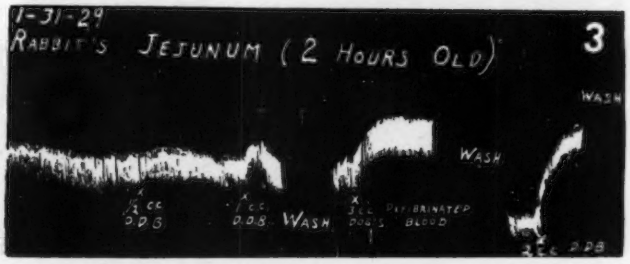
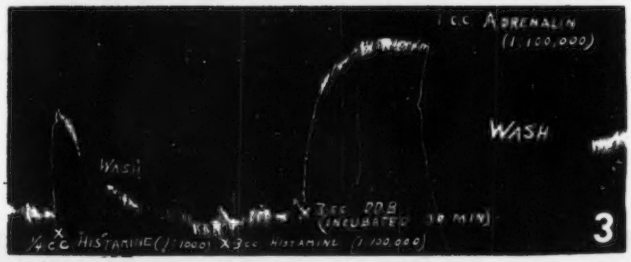
a fall in blood pressure on intravenous injection. Cannon (1923), in his book on traumatic shock, made the suggestive remark that the injection of blood in its preclotting phase causes a great fall in blood pressure, and intimated that this may be concerned with shock, a suggestion which Herrick and Markowitz also made. The latter workers found that defibrinated blood, when perfused through the isolated mammalian heart, is extremely toxic, in contradistinction to Ringer-Locke's solution; and also that the same blood, when passed through the lungs, is detoxified. With this observation in mind, I undertook a study of defibrinated blood from the point of view that perhaps it not only brings about a fall in blood pressure, as does histamine, but also that it may be an intensely stimulating agent for smooth muscle in general, again like histamine. Of the many theories of shock, a popular one has been that it is the result of extreme contraction of the arteries with (in some obscure manner) a consequent fall in blood pressure. Be that as it may, I have found that in defibrinated blood there is a substance which brings about extreme contraction of blood vessels and smooth muscle in general, and that also brings about a fall in blood pressure on intravenous injection. This work is the subject of the present communication.

METHODS. The small intestine of the rat, the guinea pig and the rabbit were studied, as well as the uterus of the virgin guinea pig and the suitably preserved carotid artery of the calf. These were suspended in the usual manner in Ringer-Locke's solution and the preparation was put in the Dale (1912–13) water bath. The recording lever magnified four times the contractions of the intestine and the uterus, and eleven times the contractions of the arterial strip. The technic of Lewis and Koessler (1927) was followed in the work with the arterial strip. This consists of attaching a spiral arterial strip, about 2 mm. wide, to the recording lever and giving it a preliminary stretching with a weight of 20 grams for ten minutes in Ringer-Locke's solution at 38 to 40°C., after which it is weighted with only 2 grams. When a steady base line is attained, the drug under investigation is added. The capacity of the bath in which the material for study was suspended was 45 cc.

RESULTS. The addition of fresh, defibrinated dog's blood to the medium in which the carotid of a calf was suspended brought about definite contraction (fig. 1). This has been shown previously by many others. However, the result was not nearly so uniform or clear-cut as the contraction that invariably was observed when defibrinated blood was added to a piece of suspended small intestine (figs. 2, 3, 4 and 5). A concentration of one part of defibrinated blood to fifty parts of Locke's solution clearly brought about contraction of a piece of intestine of a rat. The uterus of a virgin guinea pig gave similar results (fig. 6) although, on the whole, the technic was not applied as conveniently as when intestine was used. As







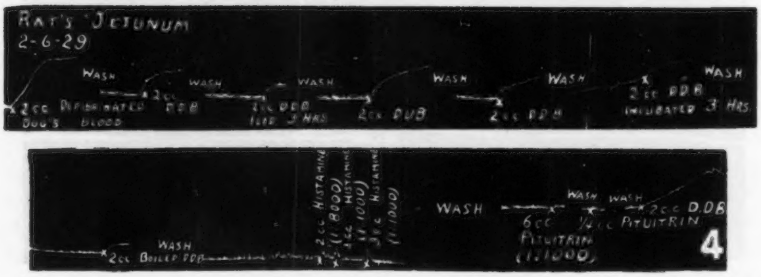


Fig. 1. Contraction of the excised carotid artery of the calf produced by defibrinated blood in a dilution of 1:50.

Fig. 2. Marked contraction of the ileum of the guinea pig produced by defibrinated blood in a dilution of 1:100.

Fig. 3. Contractions of the jejunum of the rabbit produced by varying concentrations of defibrinated blood and by histamine. Marked relaxation was produced by epinephrin. *D.D.B.* represents defibrinated dog's blood.

Fig. 4. Contractions of the jejunum of the rat produced by defibrinated blood. The effect of incubation, of refrigeration and of boiling of the blood is illustrated. Pituitrin and histamine did not cause contraction.

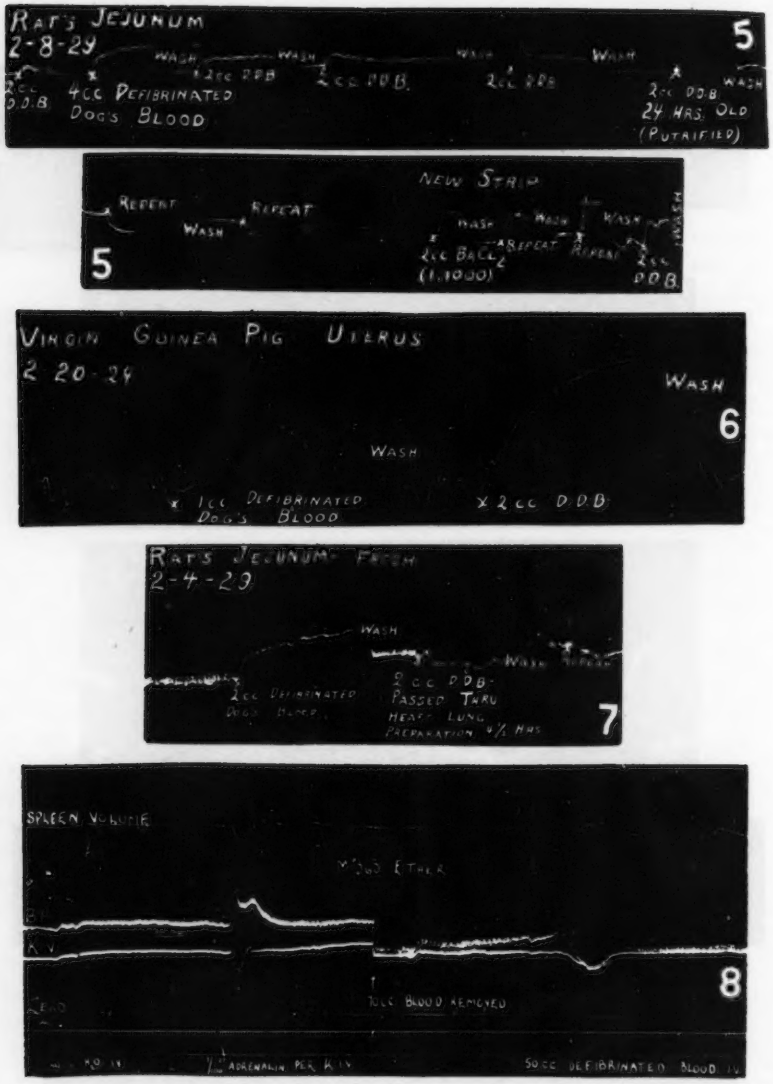


Fig. 5. In the first part of this tracing smooth muscle was exposed to defibrinated blood which had undergone putrefaction; the muscle did not contract. In the latter part of the tracing, the effect of barium chloride is also shown.

Fig. 6. Contractions of the uterus of the virgin guinea pig produced by defibrinated blood in dilutions of 1:50 and of 1:25.

Fig. 7. Contractions of jejunum of rat produced by defibrinated blood. After the defibrinated blood had been passed through the heart-lung preparation it no longer possessed the ability to cause contractions of smooth muscle.

Fig. 8. The fall in blood pressure and renal volume, with slight expansion of the splenic volume, accompanying the injection of defibrinated blood into a dog anesthetized with ether. *B.P.* represents blood pressure; *K.V.* kidney volume, and *100 mm.* the height of a column of mercury.

might be supposed from the work of Starling and Verney (1924–1925), and of Herrick and Markowitz, repeated passage of the defibrinated blood through the lung of a heart-lung perfusion preparation is followed by the diminution or disappearance of this stimulative property of the defibrinated blood (fig. 7). Incubating or refrigerating a specimen of defibrinated blood for two hours was without influence on this property (fig. 4). Blood that was allowed to stand at room temperature for twenty-four hours and that had become putrid was substantially without activity on a strip of intestine, causing a slight relaxation if anything (fig. 5). The fluid from a sample of blood that had been boiled gave only a very small reaction (fig. 4).

In order to calibrate this property of defibrinated blood against some known substance, the action on smooth muscle of defibrinated blood, histamine, pituitrin and barium chloride was compared. Histamine caused marked contraction of the intestine of the rabbit (fig. 3). However, the intestine of the rat gave no contraction with either histamine or pituitrin (fig. 4). The most convenient substance against which to assay the defibrinated blood was found to be barium chloride. In concentrations of 1:1000, this produced contraction of the intestine of the rat equal to that caused by the same volume of defibrinated blood (fig. 5).

COMMENT. These experiments bring out the fact that there is present in defibrinated blood a substance which causes intense contraction of smooth muscle; the contraction of the intestine of the rat that is induced by this substance is equivalent to that induced by solution of barium chloride 1:1000. Although the vasoconstrictor action of defibrinated blood and of blood and blood serum has been known for years, it has generally been supposed to be specific for the smooth muscle of blood vessels. The experiments reported in this paper indicate that the reaction is general for smooth muscle and more general than the reaction obtained with histamine.

Brodie pointed out that the intravenous injection of serum into a cat gives a marked fall in blood pressure, and numerous other observers have confirmed this result. It was considered wise to perform a similar experiment, by injecting defibrinated blood and noting its effect on blood pressure; after plethysmographs had been successfully applied to the spleen and to the left kidney, 70 cc. of blood were removed from the femoral artery of a dog. This blood was defibrinated, strained through gauze, warmed, and reinjected. Instead of a rise in blood pressure, there was a definite fall (fig. 8). It seems likely that, considerable as the fall in blood pressure was, it would have been much more marked if the active substance could have been injected by itself, since the administration of such a volume of blood necessarily would mask much of the depressor effect.

Two facts demonstrate that this active principle in defibrinated blood is not histamine. It has a much greater action on the intestine of the rat

than has histamine; it is destroyed by boiling and by standing at room temperature, whereas histamine is stable under these conditions.

Janeway, Richardson and Park (1918) have proved that the substance responsible for the vasoconstrictor action of defibrinated blood has its source in the disruption of platelets. It remains to be seen whether the depressor action of defibrinated blood has an identical source. This will be discussed in a subsequent paper from this laboratory. The possible relationship that such a depressor substance might have to shock is obvious. The factors which are conducive to the development of traumatic shock are identical with the factors that would bring about disruption of platelets.

SUMMARY

The addition of defibrinated blood to the perfusing fluid of the isolated intestine of the rabbit, guinea pig and rat is followed by marked contraction; the contraction of the intestine of the rat corresponds with that produced by the addition of an equal quantity of 1:1000 barium chloride. Although defibrinated blood causes contraction of a segment of carotid artery, and of a suspended uterus of a virgin guinea pig, the most convenient test object for its action on smooth muscle is the intestine of the rat. The substance responsible for the contraction is not present when the defibrinated blood is repeatedly passed through the lung. It is destroyed by boiling and by standing at room temperature. Its relation to histamine is considered, and the suggestion is made that the substance responsible for the phenomena demonstrated by the test objects may be a factor in the production of traumatic shock, since the intravenous injection of defibrinated blood is followed by a definite fall in blood pressure.

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A COMPARISON OF THE GLYCOGENOLYTIC RESPONSES TO EPINEPHRIN ADMINISTERED BY THE SUBCUTANEOUS AND INTRAVENOUS ROUTES

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It is well known that intravenous injections of epinephrin are less effective than subcutaneous in provoking glycosuria. The diminution of glycosuria is not due to the momentary antidiuretic effect for not only is there no compensatory glycosuria following, but the blood sugar itself shows a diminished response. That this is not due to any change undergone by the epinephrin during absorption is shown by the fact that when the rate of absorption is increased, e.g., by dividing and injecting in several places at once, the response becomes diminished and resembles more closely that from intravenous administration (Kleiner and Meltzer, 1913). The usual explanation advanced is that the intravenous dose produces such marked vasoconstriction that the amount of epinephrin actually reaching the liver is notably diminished. This explanation is based on two unverified assumptions, viz., *a*, that the degree of glycogenolysis is proportional to the amount of epinephrin reaching the liver, an assumption which will be discussed later in this paper; and *b*, that the vasoconstriction in the splanchnic area produced by epinephrin is sufficient to prevent its reaching the liver in sufficient quantity. This second assumption appears to me to be open to criticism. To prove it, it would not suffice to show that insufficient epinephrin reached the vessels within the liver to produce contraction, for it is generally agreed that hyperglycemia may be produced by doses too small to produce a rise of blood pressure. Data on the portal system are inconclusive, but it has been shown by Bodo and Marks (1928) that epinephrin reaching the liver through the hepatic artery is effective in causing breakdown of glycogen. The experiments of Burton-Opitz (1912) *inter alia* show that sufficient epinephrin enters the hepatic artery to produce vasoconstriction in the area supplied by it. These considerations render any explanation of the difference in effect between subcutaneous and intravenous administration based on vascular changes highly improbable.

It seemed possible, however, that an explanation might be arrived at by studying the response to doses of different magnitude. This has

already been done for the vasomotor effects of epinephrin by Lyon (1923) and Wilkie (1928). Moreover since Clark (1926) has studied not only the effect of varying the dose of acetyl choline, but also its antagonism by atropine, and since the antagonistic effects of epinephrin and insulin on the blood sugar have been studied (Eadie and Macleod, 1923), it was felt desirable to complete this by studying the effect of varying the dose of epinephrin alone on the blood sugar.

METHODS. The animal used was the rabbit. Blood was obtained from the ear vein, and the blood sugar was estimated in duplicate samples by the method of Hagedorn and Jensen.

Rabbits were starved for 48 hours, then after a normal blood sugar sample was taken, epinephrin was given. When given subcutaneously the volume of the 1:1000 solution used was made up to 1.0 cc. with 0.7 per cent sodium chloride before injection. In the case of intravenous injections it was made up to 20 cc. and injected, usually into the leg vein, sometimes into the ear vein. The injection was given at such a rate as to require two minutes for completion. After subcutaneous injection blood samples were taken in $\frac{1}{2}, 1, 1\frac{1}{2}$ and $2\frac{1}{2}$ hours, after intravenous administration in 5, 10, 30 and 45 minutes. In plotting results the figure given as "rise" was obtained by subtracting the initial value of the blood sugar from the maximum obtained over these periods, both being expressed as milligrams per 100 cc. Substantially the same results are given by other methods of computing the rise, viz., using in place of the maximum the value obtained at a definite interval of time after injection, or averaging the values at several different intervals as is done in the assay of insulin.

DISCUSSION OF RESULTS. Let us first consider the results obtained after subcutaneous injection. In spite of considerable individual variation the relation between dose and effect appears to be roughly linear. It is, of course, possible that these rather wide variations are concealing a certain degree of curvature; indeed it is highly probable that if higher doses could be employed without toxic effects the curve would flatten out. However, the general trend of results is in line with those of Clark and Wilkie cited above. At least my results are not sufficiently precise to show any difference from theirs.

A word may be said here with regard to the antagonism between epinephrin and insulin in comparison with the results of Clark on the choline-atropine antagonism. Clark uses the equation

$$K\frac{x}{x'} = \frac{y}{100 - y}$$

where x is the dose of one drug, x' that of the other, y is the effect as percentage of the maximum possible and K is a constant. A direct com-

parison is difficult since it is impractical to reckon the effect of insulin in terms of maximum. However, it can easily be shown that this is immaterial in determining the shape of the curve; it involves the substitution for y, the percentage value, of a new variable y', the actual value, and since the maximum is a constant y is related to y' in this way; y = constant

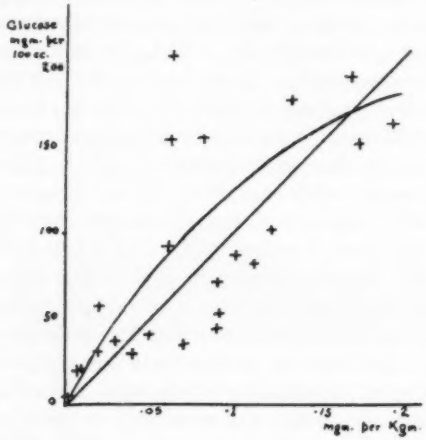

Fig. 1. The effect of subcutaneous epinephrin on the blood sugar of the rabbit. The abscissa gives the dose and the ordinate the resulting increase in blood sugar.

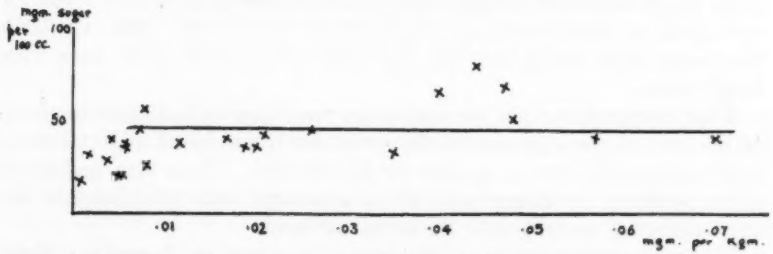

Fig. 2. The effect of intravenous epinephrin on the blood sugar of the rabbit. The abscissa gives the dose and the ordinate the resulting increase in blood sugar.

$\times y'$ and the sole effect is to change the constants of the original equation. This can have no effect on the shape of the curve. Clark's equation, if one makes x' constant, is identical with that for the dissociation curve of a weak acid, and when x is plotted against y, one obtains a hyperbola. In the case of insulin and epinephrin the same dose of the latter was used throughout, i.e., x' was constant. Reference to the curve published in

the paper cited (p. 294) shows that it is actually a hyperbola. Thus there is no reason to believe that the quantitative effects of subcutaneous epinephrin on the blood sugar differ from those on the blood pressure or from the effect of choline on muscle.

Turning now to the results of intravenous administration we find an entirely different picture (fig. 2). In the first place it may be noted that individual variations are about as great as after subcutaneous administration which probably indicates that irregularity in the rate of absorption in the latter case is negligible. However, the striking thing is that there seems to be little or no increase in effect with increase of dose. With small doses (say up to 0.02 mgm./kgm.) the results of intravenous administration are about the same as those of subcutaneous; above this the effect of the latter steadily increases while that of the former remains constant. It is, of course, impossible to give such large doses intravenously on account of the toxic effect, but there is no indication that larger doses would produce any greater result. Doses below 0.01 mgm./kgm. appear to produce a graded effect, but individual variations are so great that one cannot be certain whether even here there is any increase with increasing dose. At this point it seemed of interest to know whether similar results could be obtained in perfusion experiments which were accordingly carried out. Before describing these, however, a word may be added about time relations. After subcutaneous administration in about 50 per cent of cases the maximum blood sugar was found in 1½ hours. With smaller doses it was found more frequently to come earlier, viz., in 1 hour, but never as early as the half-hour. With larger doses it showed a certain tendency to be delayed sometimes as long as 2½ hours. E. L. Scott (1926) similarly has found with insulin that the maximum effect tends to be later with larger doses.

After intravenous doses the maximum was found in 5 minutes in about 50 per cent of cases; in most of the remainder it was found in 10 minutes; only occasionally was it as late as 40 minutes. There was perhaps a slight tendency for larger doses to be associated with later maxima but this was not so marked as in the preceding cases.

PERFUSION EXPERIMENTS. The animal used was the large frog, Rana catesbiana, of about 300 to 500 grams' weight. The method was essentially that of Fröhlich and Pollak (1914). After pithing, the inflow cannula was inserted into the abdominal vein, and a mass ligature was placed around the abdominal vessels including the portal vein. The outflow cannula was introduced into the sinus venosus and another ligature separated this from the heart and the veins of the anterior part of the body. Using a pressure of 3 to 4 inches of water the liver was perfused with Hamburger's modification of Ringer's solution as given in 1922. The outflow during 15 minute periods was collected, measured and its sugar content estimated

by the Hagedorn-Jensen method. Epinephrin was added to the perfusion fluid just before it entered the cannula by means of a syringe and needle.

A preliminary experiment showed that the first few samples contained a large amount of sugar—a washing out effect. After that the loss of sugar by the liver was almost constant provided one avoided manipulating the liver, and if a constant flow was maintained.

Figure 3 shows a typical result. Here, although the second dose was four times the first, the amount of sugar liberated was practically the same in both cases. That this is not due to there being no longer sufficient glycogen in the liver to produce a full response was shown by analyses for glycogen after the experiment had been completed. This result is in full agreement with the results of intravenous administration in rabbits.

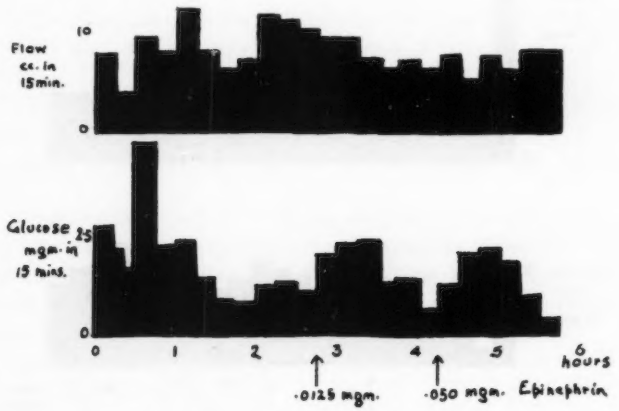

Fig. 3. Perfusion of frog's liver: the upper diagram gives the rate of flow of the perfusion fluid, the lower the amount of glucose given off.

Incidentally attention may be directed to the rate of perfusion. (See especially fig. 4.) Its constancy shows that alterations in the sugar output are not due to alterations in the rate of flow or to a consequent development of acidity as has been suggested.

These experiments show that the cause of diminished response to intravenous injections is not to be found in differences in the amount of epinephrin reaching the liver. The other difference between the two modes of administration lies in the time factor. Subcutaneous injections mean that absorption takes place over a longer period and consequently there is a continuous supply of the drug to the liver for some time. The stimulus to the liver caused by intravenous administration on the other hand is very brief, for it is well known that epinephrin disappears very rapidly

from the circulating blood. That it is not destroyed in the tissues before absorption is shown by the work of Fornet (1922).

To demonstrate this the frog experiment was modified. The first dose was of 0.6 mgm. and before the effect of this had completely worn off a second dose of 0.015 mgm. was given. Fifteen minutes after this the sugar had risen to a greater height than it had in the corresponding period after the first dose. It then began to fall but a third dose of 0.015 mgm. half an hour after the second, raised it to still greater heights. A fourth dose, 15 minutes later, seemed to have little effect and it appears that in the frog this interval is too short to allow the second dose to produce its effect.

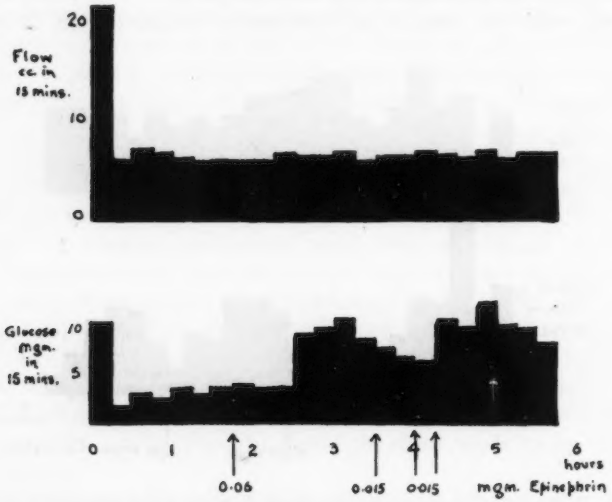

Fig. 4. Perfusion of frog's liver: the upper diagram gives the rate of flow of the perfusion fluid, the lower the amount of glucose given off.

In the mammal, of course, one would expect to find a second dose effective after a much shorter interval of time.

As a result of these experiments the following picture of the mode of action of epinephrin in producing glycogenolysis presents itself. No matter how administered, epinephrin arriving by way of the blood stream in the liver stimulates the sympathetic nerve endings. The result of this stimulation is that the chemical or physical configuration of the liver cell is so altered as to release a definite amount of glycogen.[1] This is probably

[1] I consider it much simpler to suppose this rather than that enzyme is liberated. In the latter case one must suppose that another process then occurs by which the enzyme is again removed from the sphere of action, whereas here the glycogen liberated is disposed of by hydrolysis.

an "all-or-none" effect as far as the nerves are concerned, i.e., each nerve ending when stimulated always liberates the same (rather limited) amount of glycogen no matter how strong the stimulus is provided it is effective. The amount of glycogen liberated will probably depend on the amount stored and perhaps on other factors, thus producing individual variation. The glycogen liberated is then gradually hydrolyzed and the glucose given off to the blood stream. After intravenous administration this is all that happens. But after subcutaneous administration further amounts of drug reach the liver and more glycogen is released so that the output of sugar goes on much longer (cf. the times for maximal blood sugars given above) and the hyperglycemia reaches much higher levels.

SUMMARY

1. The hyperglycemic response to subcutaneous epinephrin in the rabbit increases with the dose in a way that corresponds roughly with the pressor response.

2. With the possible exception of very small doses the response to intravenous epinephrin is independent of the dose.

3. The same independence is found to hold when the frog's liver is perfused.

4. The usual explanation for the failure of intravenous doses of epinephrin to provoke as great a hyperglycemic response as subcutaneous doses based on vasoconstrictor effects is shown to be based on improbable assumptions, and it is suggested that duration of stimulation rather than intensity is the deciding factor.

5. Adrenalin-insulin antagonism is discussed in the light of the antagonism between choline and atropine.

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THE VARIATIONS IN THE ACIDITY OF THE GASTRIC JUICE DURING THE SECRETORY PERIOD

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During a study of the possible functional activity of a third secretory element in the gastric glands, the so-called "mucoid cells," "Nebenzellen" or "Zwischenzellen" (1) in dogs, I became interested in the problem of the regulation of the acidity of the gastric juice. This problem was recently discussed by Maclean, Griffiths and Williams (2), who came to the conclusion that the regurgitation of alkaline duodenal juices does not play an important part in the regulation of gastric acidity. According to them, a neutral fluid is secreted by the gastric glands at the end of the digestive period which reduces the gastric acidity to the fasting level. Since the results obtained in our laboratory from experiments on dogs could not be explained from the point of view expressed by these authors, it seemed desirable to report them here.

METHODS. Two dogs were used, one with a gastric fistula and esophagotomy, the other with an isolated stomach pouch. The latter was operated on for a Pavlov pouch (in December, 1928), but subsequent observations showed that the secretory nerves in the bridge had degenerated and we had to deal with a Heidenhain pouch. Thus both the phases of gastric secretion—the reflex phase and the chemical phase—could be studied separately.

Tests were made to determine the free acidity (Töpfer's solution), total acidity (phenolphthalein) and total chlorides (van Slyke's method) in the gastric juice. The amount of mucus in the samples of juice was also ascertained. This was done either volumetrically, samples of juice being centrifuged in graduated tubes and the amount of mucus expressed in volume per cent., or the samples of gastric juice were filtered through alundum crucibles and dried in an oven at 105°C., the dry weight of the mucus being then determined.

Results of experiments on a dog with œsophagotomy and gastric fistula. Table 1 shows the results following 10 minutes' sham feeding with meat. The secretion continued for $2\frac{1}{4}$ hours, gradually diminishing. The content of total chlorine in the juice was very constant, a fact formerly observed by Rosemann (3) in dog's gastric juice after sham feeding. The total

acidity underwent some variations during the experiment. At the beginning it was somewhat lower (0.51–0.52 per cent HCl). This coincided with the presence of bile in the first three samples of juice. As soon as the juice became water-clear, the total acidity rose (0.62–0.56 per cent HCl), although the amount of juice secreted was then greatly diminished. As it diminished further, the total acidity continued to fall. The lowest total acidity recorded in this experiment was 0.43 per cent HCl. In the first part of the experiment (samples 1 to 6) the free acidity ran practically parallel with the total acidity. Beginning with the 7th sample there is a sharp fall in the free acidity (0.29 per cent HCl). For an explanation of the moderate fall in the total acidity and the abrupt fall in the free acidity we must consider the column "mucus, volume per cent." There is a gradual increase in the amount of mucus admixed with the juice. Insignificant in the first period of secretion, it reached, with certain variations,

TABLE 1

Dog with esophagotomy and gastric fistula

April 22, 1929. Gastric secretion after 10 minutes sham feeding with meat

TIME IN 15 MINUTES	AMOUNT	FREE HCl	TOTAL HCl	TOTAL Cl′ AS N/10	MUCUS VOLUME	REMARKS
	cc.	*per cent*	*per cent*		*per cent*	
1	28.0	0.43	0.51	1.36	1.0	Bile
2	22.0	0.44	0.52	1.36	1.7	Bile
3	12.0	0.44	0.52	1.43	3.8	Bile
4	4.3	0.51	0.62	1.33	23.2	Clear
5	4.1	0.53	0.62	1.36	29.2	Clear
6	4.3	0.47	0.56	1.4	45.0	Clear
7	3.4	0.29	0.43		35.0	Great amount of mucus
8	1.5	0.29	0.47		50.0	Great amount of mucus
9	2.2	0.29	0.51		77.0	Great amount of mucus

a very high proportion in the last samples of juice (from 35 to 77 volumes per cent). This one factor is sufficient to explain the fall in the total and especially in the free acidity of the last samples of the juice. It would, however, be inaccurate to deny completely in this experiment the different distribution of Cl between HCl and NaCl in the sense of Rosemann (3) in separate samples of the juice. This is indicated by a comparison of samples 7 and 8 with the 9th sample of juice, in which the amount of mucus reached its maximum (77 vols. per cent), but in spite of this the total acidity rose a little. In other words more chlorine appeared in the form of HCl than before, which might be due to a fresh stimulus transmitted along the vagus nerve from the hemispheres. (The experiment lasted $2\frac{1}{4}$ hours.)

Thus during the reflex phase of gastric secretion nothing in the nature of a neutral fluid is produced by the gastric glands. The gastric juice throughout the whole secretory period is completely acid.

The two following experiments on the same dog show the influence of chemical stimuli such as histamine (subcutaneous injection) and alcohol (per rectum) on a stomach with innervation intact. The first (reflex) phase in these experiments was eliminated.

In table 2 we see again that the secretion of gastric juice, activated in this case by subcutaneous injection of 0.75 gram histamine, was to the

TABLE 2

Dog with esophagotomy and gastric fistula

May 2, 1929. Gastric secretion after subcutaneous injection of 0.75 mgm. histamine

SAMPLE NUMBER	TIME	QUANTITY	FREE ACIDITY IN PER CENT HCl	TOTAL ACIDITY IN PER CENT HCl	TOTAL Cl' AS N/10	MUCUS VOLUME	REMARKS
	minutes	*cc.*				*per cent*	
1	5	4.5	0.33	0.40	1.16	0.2	Bile
2	5	9.8	0.40	0.45	1.2	0.2	Bile
3	5	15.0	0.40	0.44	1.28	0.6	Clear
4	15	48.0	0.45	0.51	1.4	0.2	Clear
5	15	44.0	0.51	0.58	1.4	0.2	Clear
6	15	28.5	0.48	0.54	1.4	0.3	Clear
7	15	8.8	0.46	0.50	1.28	0.1	Clear
8	60	0.7	0.36	0.40		7.0	Mucus present
9	60	0.5					Mucus only

TABLE 3

Dog with esophagotomy and gastric fistula

May 5, 1929. Gastric secretion after introduction of 200 cc. 10 per cent alcohol solution per rectum

TIME IN 15 MINUTES SAMPLE	AMOUNT	FREE ACIDITY IN PER CENT HCl	TOTAL ACIDITY IN PER CENT HCl	TOTAL Cl' AS N/10	MUCUS VOLUME	REMARKS
					per cent	
1	32.0	0.34	0.44	1.2	0.3	Clear
2	25.0	0.44	0.49	1.2	0.4	Clear
3	11.5	0.44	0.47	1.23	0.8	Clear
4	6.2	0.44	0.49	1.2	1.6	Clear
5	2.3	0.40	0.45		4.3	Clear
6	1.2	0.36	0.40		4.1	Clear

very end of the experiment perfectly acid. The variations in the acidity were due partly to the admixture of bile (samples 1 and 2), partly to the diminishing rate of secretion of the juice and its neutralization by the mucus (sample 8). It is very remarkable that histamine does not activate the production of mucus in large amounts. In spite of the extremely low content of mucus the total acidity in the clear samples of juice was some-

what lower than in the case of sham feeding. This suggests that different secretory mechanism is involved in the case of nervous action and in the case of histamine.

TABLE 4

Heidenhain's pouch dog

Secretion of gastric juice after meals of milk, meat, and bread and milk

SAMPLE NUMBER	TIME	AMOUNT	FREE ACIDITY IN PER CENT HCl	TOTAL ACIDITY IN PER CENT HCl	REMARKS
		Experiment February 7, 1929			
	minutes	*cc.*			
0	50	0.7		Slightly acid	Mucus. Before meal
1	60	2.85	0.31	0.38	600 cc. milk given
2	60	4.35	0.42	0.49	
3	60	1.25	0.38	0.45	
4	60	1.3	0.13	0.18	
5	60	4.0	0.45	0.54	300 cc. milk given
6	60	3.5	0.38	0.48	
7	60	1.7	0.36	0.45	
		Experiment February 8, 1929			
0	60	0.4		Slightly acid	Mucus. Before meal
1	60	4.0	0.42	0.49	200 grams minced meat given
2	60	2.0	0.40	0.43	
3	60	2.15	0.36	0.45	
4	60	2.7	0.38	0.47	
5	60	2.7	0.32	0.38	
6	60	1.9	0.31	0.40	
		Experiment February 15, 1929			
00	60	0.5		Slightly acid	Mucus. Before meal
0	30	0.2		Slightly acid	Mucus. Before meal
1	30	5.0	0.22	0.29	150 grams bread powder and 600 cc. milk given
2	30	3.0	0.40	0.51	
3	60	1.4	0.40	0.44	
4	60	0.8	0.36	0.40	
5	60	0.7	0.26	0.36	
6	60	0.8	0.25	0.32	
7	60	0.95	0.29	0.36	

Analogous results were obtained when a 10 per cent alcohol solution was introduced per rectum (table 3). Throughout the experiment the gastric juice was acid. The content of mucus, as in "histamine" juice, was very low; and although the secretion was abundant at the beginning of the experiment, the total acidity was lower than in the case of histamine and

much lower than after sham feeding. It is interesting to note that the Cl content of "alcohol" juice was somewhat lower than in the other kinds of gastric juice.

Results of experiments on a dog with a Heidenhain pouch. In table 4 are given results following three experimental meals of milk, meat, and bread powder and milk (the dog refusing to eat bread). The experiments were performed about two months after operation, before the secretion of the isolated pouch became diminished, as it did later. No systematic determinations of the amount of mucus in the juice were made at that time.

The characteristic feature of all these experiments is that the acidity fell in the second hour of diminished secretion (cf. expt. Feb. 7, samples

TABLE 5

Heidenhain's pouch dog

Secretion on milk and milk with alcohol

SAMPLE NUMBER	TIME	AMOUNT	FREE ACIDITY IN PER CENT HCl	TOTAL ACIDITY IN PER CENT HCl	TOTAL Cl' AS N/10	MUCUS VOLUME	REMARKS
Experiment April 18, 1928							
	minutes	*cc.*				*per cent*	
1	60	2.7	0.23	0.29	1.4	1.8	Milk 600 cc.
2	60	6.0	0.43	0.47	1.4	0.8	
3	60	1.4	0.36	0.43		3.5	
4	60	1.0	0.18	0.22		25.0	
5	60	1.0		0.09		81.9	
Experiment April 16, 1929							
1	60	8.4	0.36	0.44	1.4	1.2	600 cc. milk with 15 cc. 95 per cent alcohol
2	60	8.0	0.43	0.49	1.4	3.7	
3	60	5.3	0.38	0.43	1.4	9.4	
4	60	0.6	0.05	0.07		25.0	
5	60	0.95		0.07		26.0	

3 and 4, and 7; expt. Feb. 8, samples 5 and 6; expt. Feb. 15, samples 5, 6 and 7). Another point of importance is that the fall in the free acidity was more pronounced than the fall in the total acidity, which indicates that the primary factor in diminishing the acidity of the juice is the formation of protein salt from the hydrochloric acid and not the secretion of Cl chiefly in the form of sodium chloride.

When the stimulus at the end of the experiment was very weak (cf. table 4, expt. Feb. 7, sample 4), the stomach pouch—it would not be quite accurate to say "the gastric glands"—produced a slightly acid or neutral fluid containing a large amount of mucus. Thus the activity of the stomach gradually reverted to the state in which it was before the meal.

When the stimulus was stronger (cf. expt. Feb. 15—bread and milk), in spite of a very scanty flow of gastric juice from the pouch (0.7 to 0.95 cc. per hour), the gastric glands secreted for hours a distinctly acid juice. Neither in these nor in other experiments, however, did the isolated pouch appear to produce any appreciable amount of "neutral" fluid able under other conditions to dilute and change the reaction of the gastric contents.

In table 5 examples are given of feeding with milk and with milk and alcohol. In this set of experiments the volume per cent of mucus was determined. The amount of mucus was increased towards the end of the experiment. This increase was not so great in the case of the milk and alcohol as in the case of the pure milk. This might be due to the specific action of the alcohol, which did not cause, as we have seen above, an appreciable production of mucus. A fall in the acidity of the gastric juice coincided with the increased amount of mucus. In both cases a low acidity was observed when the pouch secreted a very insignificant amount of juice (0.6 to 1 cc.), rich in mucin. The experiments with milk (cf. table 5 and also table 4) give the impression that the fall in the acidity of the gastric juice after a meal of milk is more abrupt than after a meal of meat or bread. This fact deserves further investigation.

None of the experiments on the dog with the isolated gastric pouch gave any evidence that the acid secretion was replaced by a neutral secretion. At the end there was only a scanty flow of fluid very rich in mucus. It was small in quantity when compared with the amount of highly acid gastric juice secreted during the preceding hours, so that the reduction in the acidity of this secretion, if occurring in the main stomach, could not be explained in this way.

Discussion. The results obtained in this investigation will be compared only with the results obtained by MacLean, Griffiths and Williams from experiments on dogs with a Pavlov pouch. The experiments of MacLean and his co-workers on man will not be considered, since we have no corresponding material, and also because the existence of the "Verdünnungssecretion" which these authors advocate was formerly disproved by Lönnquist (4), using Pavlov pouch dogs with stomach disconnected from duodenum. From his experiments in which he introduced solutions of NaCl of different concentrations into the main stomach disconnected from the duodenum, and from observation of the gastric secretion from a Pavlov pouch, it was clear that two facts must be discriminated, namely, the secretion by the Pavlov pouch under these conditions of perfectly normal gastric juice with a very high acidity, and the changes in the acidity of the gastric contents due to irritation of the gastric mucous membrane by the solution, secretion of pyloric juice, etc. Moreover Rasenkov (5) showed recently that the monovalent anions of different salts activate the secretion of gastric juice, and the bi-valent anions chiefly stimulate

the production of gastric mucus. Therefore in introducing different chemical agents into the stomach as MacLean and others have done, one must take into consideration not only the actual secretion of gastric juice but also the gastric mucus which will be produced and which may neutralize the juice.

Turning to our experiments on a dog with esophagotomy and gastric fistula, we see that the total acidity of the gastric juice secreted under nervous influence undergoes some slight variations. The variations in the free acidity are more pronounced. Its low standing coincided with the presence of bile in the juice and more especially with the proportional increase of mucus in the juice. Practically identical relations were found when the gastric glands were stimulated by histamine or alcohol. In all these cases the fall in the acidity was especially pronounced at the end of the experiment. While admitting the important part which mucus plays in the neutralization of the gastric juice, and the fact of its partial dissolution in the acid of the juice (6), we think that a second factor should be recognized, namely, larger secretion of Cl in the form of NaCl at the end of the secretory period (3). Besides this the possibility of greater activity of the mucoid cells at this time should be borne in mind (1). In none of our experiments, however, did the normally innervated stomach secrete a fluid of low enough acidity or sufficient amount to dilute the acid gastric contents.

Our experiments on a Heidenhain pouch dog also showed that the fall in the total acidity occurred only at the very end of the secretory period, when the amount of juice was quite insignificant and contained a large amount of mucus. Another interesting feature of these experiments was that during two successive hours of low secretion a really great fall in the acidity occurred only in the second hour. This indicates again that one should be very careful in speaking of a "secretion" of gastric juice of low acidity. The possibility that the juice may become neutralized in flowing over the walls of the gastric mucous membrane, has to be taken into consideration. Furthermore, in Heidenhain pouch dogs, especially as time advances after the operation, the pouch produces less secretion and the secretion is less acid than in Pavlov pouch dogs. Although Orbeli (7) thought that the lower acidity of the gastric juice in the Heidenhain pouch dog depends on the smaller quantity of juice secreted, it seems to us that a part of the mucous membrane, if wholly or almost wholly deprived of its extrinsic vagal innervation, is not likely to produce juice of as great acidity as before, since the vagus plays a very important part in the production of hydrochloric acid.

If we turn now to the data obtained by MacLean, Griffiths and Williams, working on Pavlov pouch dogs, we see that three hours after a meal of 100 grams meat the acidity had fallen to 0.230 per cent HCl (0.63 N/10 chloride); and in the case of a bread (dog biscuit) and water meal it fell to

0.222 per cent HCl (0.61 N/10 chloride) in two hours. MacLean and his co-workers do not indicate how soon after operation they began their experiments, and do not record the amount of juice secreted. They mention only (p. 80) that at the end of the digestive period "it was difficult to get sufficient fluid for quantitative analysis." The qualitative tests showed that the juice secreted at this period had only a faintly acid reaction. All this shows that in the dogs of MacLean and his co-workers the Pavlov pouch did not function quite normally. If we refer to the classical data of Chishin (8) we see that his dog with a perfect Pavlov pouch secreted for four hours after a meal of 100 grams meat. The amount of juice secreted every hour and the total acidity (phenolphthalein) in these experiments were on an average as follows: 10.5 cc.—0.538 per cent HCl; 8.6 cc.—0.560 per cent HCl; 4.8 cc.—0.547 per cent HCl; 2.4 cc.; 0.8 cc.: total 26.3 cc. Acidity of proportionally mixed juice, 0.543 per cent HCl.

Our figures for acidity after a meal of 200 grams meat were not so high as those of Chishin, but for six hours a perfectly acid juice was secreted (max. 0.49 per cent HCl; min. 0.38 per cent HCl).

The conclusions which may be drawn from all these experiments are as follows:

The acidity of the pure gastric juice undergoes definite but slight variations. This is due 1, partly to the neutralization of the gastric juice by the alkaline mucus of the surface epithelium (1); 2, partly to the varying distribution of Cl between HCl and NaCl (3); 3, partly to the secretion of mucoid fluid by the peptic glands themselves (9) (1). However, under normal conditions the gastric glands never secrete sufficient neutral or even slightly acid fluid to dilute the gastric contents and reduce their acidity. There are other factors which lower the acidity of the gastric contents after a meal.

SUMMARY

1. Nervous and chemical (histamine, alcohol) stimulations of normally innervated gastric glands in a dog activate a flow of gastric juice, in which the acidity is slightly lowered at the end of the secretory period.

2. In a dog with a Heidenhain pouch ingestion of different foods produces a flow of acid juice and finally a scanty secretion of slightly acid fluid rich in mucin.

3. The variations in the acidity of the gastric juice, which are not very pronounced under normal conditions, are attributed to several factors discussed in this paper.

4. Normal gastric glands never at any period of their activity secrete an appreciable amount of fluid with sufficiently low acidity to neutralize the gastric content .

My thanks are due to Dr. B. P. Babkin for helpful criticism during this work.

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THE SIMULTANEOUS PRODUCTION OF TWO HORMONES BY THE CORPUS LUTEUM[1]

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Since 1897 the yellow body has been regarded as a gland of internal secretion. Beard (3) and Prenant (15) believed that the corpus luteum inhibited ovulation. Fraenkel (6), in 1903, showed that the corpus luteum was essential for the nidation of fertilized ova. Leo Loeb (12), in 1909, by a different type of experiment demonstrated a sensitizing function. The secretion of the corpus luteum was shown by him to produce the maternal portion of the placenta necessary for successful nidation.

Since 1912, Iscovesco (11), Fellner (5), Frank and Rosenbloom (7), Frank and Gustavson (8), to mention only a few, have shown that the yellow body of sows, cows, etc., contained female sex hormone. More recently Pratt and Allen as well as Zondek and Aschheim (17), demonstrated the presence of the female sex hormone in the human corpus luteum.

During the last two years much additional evidence has accumulated upon the special inhibiting and sensitizing hormone found in the corpus luteum through the work of Papanicolaou (13), Hisaw (10), Weichert (16), and quite recently Allen and Corner (9), as well as Corner and Allen (4).

It appears definitely proved that a water soluble fraction of the corpus luteum inhibits the growth of ovarian follicles and likewise produces the sensitization of the uterus (experimentally demonstrated by the production of artificial placentomata and deciduomata) necessary for embedding and early growth of the fertilized ovum.

Recently we have been able to demonstrate the presence not only of a water soluble inhibitory and sensitizing fraction in the corpus luteum, but from the same batch of material have extracted, by means of a lipoid solvent, the female sex hormone, the presence of which was proved by the production of artificial estrus in castrated rats.

The following is a brief description of the chemical data. Six hundred ten grams of the pink type of corpora lutea were obtained from five

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pounds of sows' ovaries. The corpora lutea were ground with sand and extracted for twenty-four hours with acid alcohol. This alcoholic extract contains both hormones. The alcoholic extract was evaporated under reduced pressure at a temperature below 50° Centigrade to an aqueous sludge. The low temperature is necessary so as to avoid destruction of the inhibitory hormone, which in the experience of Hisaw (10) is destroyed by a temperature of 60°C. The aqueous sludge was made neutral to litmus. The female sex hormone was removed by extracting the material three times with ether. The inhibitory hormone is insoluble in ether (10). Thus two fractions were obtained; 1, an ether-insoluble, aqueous; 2, an ether fraction.

1. The aqueous fraction was purified by taking advantage of the properties of the inhibitory hormone as described by Hisaw (10). It was dialyzed for twenty-four hours through a collodion membrane which would not let congo red dye pass through its pores. The material after dialysis was filtered through an alundum crucible, evaporated down to a volume of 25 cc. under reduced pressure at a temperature below 50°C. The material was again filtered through an alundum crucible. A clear light yellow solution was obtained, each cubic centimeter of which was equivalent to twenty-five grams of corpora lutea.

The aqueous fraction was first tested by the Weichert (16) method, in which castrated rats were brought to a strong positive reaction by means of the injection of the female sex hormone, and then on two successive days the aqueous corpus luteum hormone was injected instead. Thereupon the abdomen was opened and silk threads were passed through the uterine wall. For four succeeding days these rats received 1 cc. of the aqueous corpus luteum fraction and were then killed. Macroscopically no deciduomata were found; microscopically the test for deciduomata also proved negative.

As this test was negative, Hisaw's (10) method of testing was used. Virgin guinea pigs were taken at the time of full estrus and given 1 cc. of the aqueous fraction subcutaneously. The next morning the solidity of the symphysis pubis was tested and in the three animals used, marked mobility was found twelve hours after the injection which is interpreted as a positive reaction.

2. The ether fraction was purified by means of ammonia. By this technic, described by Gustavson and Goodman (9), the cholesterol, which in bulk is the chief contaminant, is removed as the hormone is soluble in liquid ammonia, the cholesterol insoluble. The ammonia soluble fraction was taken up in oil, each cubic centimeter of olive oil containing 22 mgm. of solid.

The ether fraction was then tested on castrated rats. In doses of 22 to 50 mgm. a strong positive reaction by the vaginal smear test of Allen

and Doisy (1) was obtained. This signifies the presence of the female sex hormone in the amount which we (8) have repeatedly found in the corpus luteum of the sow and cow.

It thus is apparent that the corpus luteum, like the anterior lobe of the pituitary, is able and does elaborate two distinct and different hormones simultaneously, both of which are necessary for the complete function of the genital tract. The one we designate as the female sex hormone is secreted by the growing and mature follicle, the corpus luteum, as well as by the placenta (the three forming the gestational gland). It is the anabolic hormone. The other, the aqueous corpus luteum hormone, is the cycle inhibiting and "nidatory" hormone.

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THE EFFECTS OF BRONCHOSPASM ON THE CIRCULATION OF GUINEA PIGS

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Since Auer and Lewis (2) demonstrated that, in guinea pigs, death from anaphylactic shock is due to asphyxia resulting from bronchospasm, various phases of the circulatory response which accompanies it have been studied. Numerous observers (1–8) have investigated circulatory effects in dogs, cats and rabbits during the bronchospasm from anaphylaxis and other causes. The measurements which have been made, however, either have been isolated ones or have been accomplished by opening the chest, a procedure which destroys the effect of the all-important intrathoracic pressure.

In no instance were arterial, venous and intrapleural pressures recorded simultaneously and such measurements approach their true values only if the thorax remains closed. The experiments here recorded are the only ones carried out under those conditions. They were undertaken to explain, if possible, the clinical findings recently reported (9) that the heart remains singularly free from appreciable damage in cases of long-standing asthma. An asthmatic paroxysm in which bronchospasm occurs has long been recognized as being quite similar to the pulmonary manifestations of mild anaphylactic shock.

METHOD. Guinea pigs of 700 to 1000 grams were sensitized by an intraperitoneal injection of from 3.0 cc. to 5.0 cc. of a 1 to 2 suspension of egg white in buffered saline solution (10). The animals were allowed 15 to 20 days to become sensitive to the egg white before giving the shocking dose of 0.5 cc. to 1.0 cc.

Guinea pigs of the size described here are difficult to sensitize; for this reason the large doses of egg white were used.

The animals were anesthetized with amytal for the experiment. This was given intraperitoneally in doses proportional to 50 mgm. of the drug per kilo of body weight.

A dissection of the anterior aspect of the neck was made by an incision from just posterior to the mouth to about the middle of the sternum. The right subclavian vein was exposed to the point where it enters the chest. The trachea and left common carotid artery were dissected out.

A cannula was placed in the right subclavian vein through a nick in the vessel wall about 1.0 cm. from the place where the vein enters the chest. The cannula was then worked down the vein until it pulsated with the beating of the heart. Under these circumstances, at autopsy, the tip of the cannula was always found to be within the right auricle. All animals were autopsied in order to locate definitely the position of the cannula during the experiment.

This venous cannula was connected to a water manometer made of straight drawn glass tubing and equipped with a recording float similar to that used with mercury manometers. The float was made to fit the tubing closely enough so that water could not easily pass it, but loosely enough to offer practically no resistance to its movements. The manometer was found to be very sensitive and would indicate the slightest changes to pressure.

Physiological saline solution containing 0.7 per cent of heparin was used in this system to prevent clotting of the blood. The solution of saline and heparin was run into the system from a burette so that an accurate account of the fluid used during the experiment could be kept. The fluid entering the circulation of the animal was usually about 5.0 cc. during the entire experiment.

Another cannula was placed in the left carotid artery and connected to a mercury manometer. A 5.0 per cent solution of sodium citrate was used in this system to prevent clotting of the blood. The citrate was run into the system from a height sufficient to equal the blood pressure.

A third cannula was placed in the trachea and connected to a three liter flask (vorlage) which, in turn, was connected to a tambour so as to furnish a closed breathing system which would indicate when air entered and left the lungs. The animal was not connected to the flask until just previous to giving the shocking dose in order to avoid any possible effects of asphyxia.

A trochar connected to a tambour was placed in the pleural cavity by pushing it through a nick in the skin between the ribs. This trochar was inserted near the mid-axillary line on the right side. The procedure did not disturb the intrapleural pressure since the closed system remained intact. The tambour was standardized on the graph in order to transpose its graph to centimeters of water pressure.

These pressures were all recorded simultaneously on a kymograph so that a composite record was obtained.

After the satisfactory insertion of these cannulae, the drum was run long enough to record normal pressures. Five-tenths cc. to 1.0 cc. of the egg-white suspension was then injected into the venous cannula and washed into the circulation with the saline-heparin mixture.

With this method it was hoped to show the effect of bronchospasm on

the circulation. Three separate pressures were taken during the bronchospasm, as described above, and a fourth pressure was calculated. The pressures recorded were the intrapleural, intra-auricular and carotid artery pressures. The effective venous pressure was calculated. The effective venous pressure was taken to reflect the degree of filling of the right heart.

It is recognized that the calculation of effective venous pressures will vary, depending upon the places where the venous measurements are made and also upon the phases of the heart cycle. The effective pressures here recorded are mean pressures and were calculated according to the formula of Wiggers (11). The results arrived at represent the algebraic difference between the geometric mean of the intrapleural pressure and of the intra-auricular pressure. In none of the previous experiments stated above were geometric mean pressures recorded. As will be seen from the tracings, the pressure curves are so irregular that only by such measurements can accurate determinations of mean pressures be approached. This was done by dropping parallels from points along the curves to the zero line. Enlargements of the graphs were then made and the surface areas included between the parallels were determined by a planimeter. In addition to the above pressures, measurements were made to indicate when air was entering and leaving the chest. This was indicated on the graph as "vorlage."

RESULTS. A representative record is here reproduced.

DISCUSSION. In all, partial or complete readings were obtained in over fifty animals. These showed the following as the result of the shocking dose.

Intrapleural pressure: There is an immediate fall, sometimes slight but usually pronounced. This is attributed to the fact that during the early stages of bronchospasm, as an increasing amount of air becomes entrapped in the lungs, the lungs expand. During this phase an increasing negative pressure develops within the pleura due to the increased inspiratory effort until the lungs have assumed the inspiratory position. This is followed by a rise in pressure which at times is great and exceeds atmospheric pressure. This apparently is coincident with violent respiratory efforts to expel the air entrapped in the inflated lungs. This pressure remains elevated until death.

Intra-auricular pressure: There is usually a temporary drop coincident with that of the intrapleural pressure, followed by a rise which may reach a very high level. This elevated pressure continues and, as the heart gradually becomes adynamic and overfills, the intra-auricular pressure becomes higher still and is so recorded as the animal dies. The variations of intra-auricular pressure are attributed to the influence of intrapleural pressure on the structures within the thorax. It is possible that there is an obstruction in the pulmonary arterial system which is reflected in a back

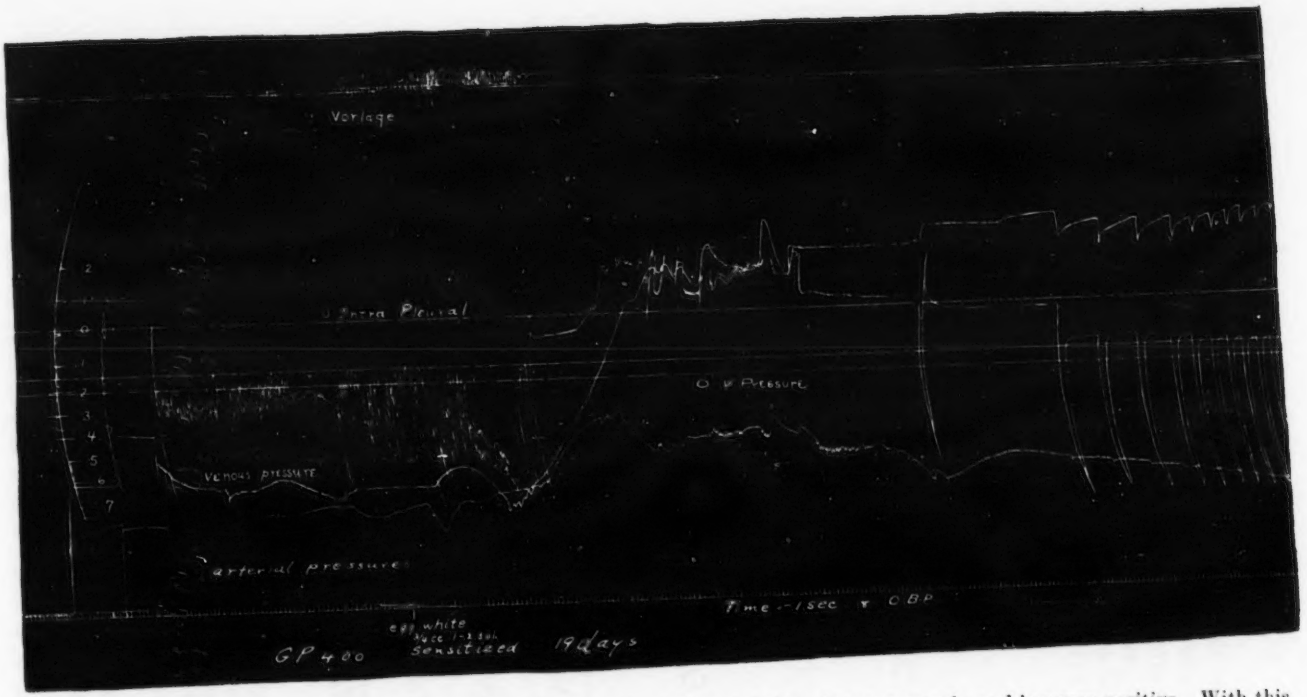

Fig. 1. After the injection of egg-white, the intrapleural pressure falls and then rises enormously and becomes positive. With this, there is a rise in both arterial and venous pressures. The vorlage at the top of the graph shows first an increase in respiratory rate, then a slowing, finally a cessation. The intra-auricular (venous) pressure line is retraced with ink.

In order to see pressure relationships more clearly, a graph of this record was made to scale. The calculated effective venous pressure is also shown on this.

pressure in the right auricle. This would contribute toward a rise in intra-auricular pressure.

Carotid pressure: The changes are by no means as pronounced as those of the other recorded pressures. There may or may not be an initial fall, or rise. This is followed by a rise which is attributed to the effect of asphyxia. As the animal dies, there is a final fall.

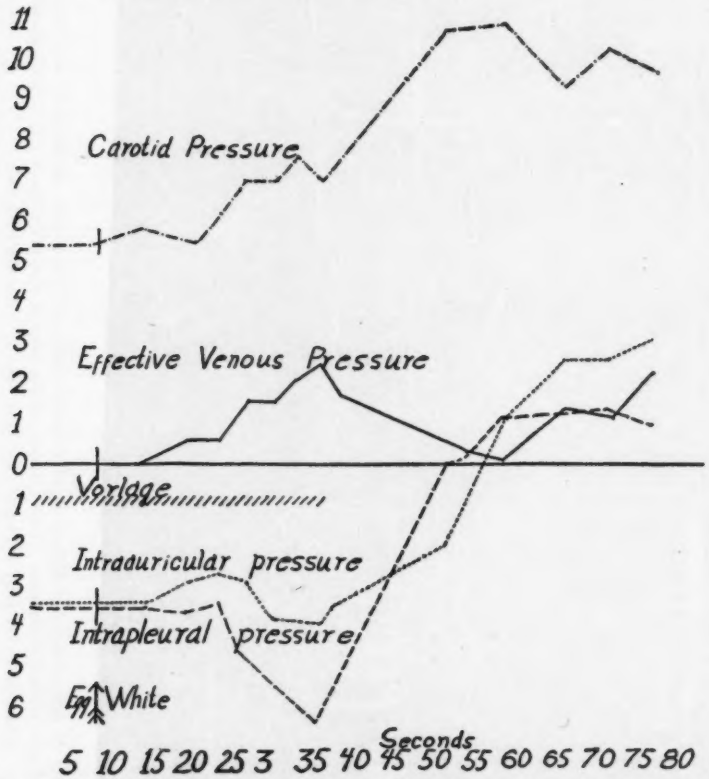

Fig. 2. This is a transposition of figure 1. Both intra-auricular and intrapleural pressures show marked elevation. The effective venous pressure rises as they become divergent and falls as they approximate each other. All pressures on the graph are recorded in centimeters of water, except carotid pressure which is in millimeters of mercury.

Effective venous pressure: Since this is a calculated pressure, it depends entirely upon the recorded pressures within the pleurae and right auricle. If these are parallel, the effective venous pressure remains a straight line; if they coincide, it equals zero; if they diverge, it rises. In all cases this

effective venous pressure showed a rise soon after the injection of egg white, especially so in the cases where the bronchospasm was fatal to the guinea pigs. At times, the effective venous pressure rose but little as the changes in intrapleural and intra-auricular pressures were only slight. In these cases the animals continued to get air in and out of the lungs during the whole experiment and did not die from bronchospasm.

SUMMARY

1. The circulatory changes resulting from bronchospasm were studied. Particular reference was made to the effective venous pressure which was taken to reflect the degree of filling of the right heart.

2. When bronchospasm was extreme there was a marked increase in the effective venous pressure.

3. When bronchospasm was not marked, the effective venous pressure showed very little change.

4. Carotid, intra-auricular and intrathoracic pressures were recorded simultaneously. The behavior of each of these is described.

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